Monitoring of Chinese Mitten Crabs (Eriocheir sinensis) on the River Dee

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North Wales Wildlife Trust

Report No 154

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Sefydlwyd Prosiect cranc mynegog Tsieineaidd (Chinese mitten crab Project) ar y Ddyfrdwy er mwyn ymchwilio poblogaethau Cranc mynegog Tsieineaidd yn nalgylch Afon Dyfrdwy. Y bwriad oedd cyflawni nifer o amcanion gan cynnwys codi ymwybyddiaeth, treialu a gwerthuso dulliau pwrpasol o fonitro a rheoli'r boblogaeth a llunio casgliadau am statws cyfredol ac i'r dyfodol y rhywogaeth hwn yn nalgylch yr Afon Ddyfrdwy.

Roedd y rhaglen waith o dan y cynllun estyn allan i greu ymwybyddiaeth yn llwyddiannus. Llwyddwyd i gysylltu ag amrywiaeth eang o grwpiau defnyddwyr yn nalgylch y Ddyfrdwy gan gynnwys cwmnïau dŵr, pysgotwyr a physgotwyr masnachol ac eraill sy'n defnyddio'r afon a'u rhybuddio o'r risgiau sy'n gysylltiol â'r cranc manegog Tseineiadd. Er hynny, mae'n anodd asesu pa mor effeithiol mae cynllun o'r fath wedi bod mewn atal lledaenu pellach. Gallai hyn fod oherwydd natur ddirfel ycranc mynegog Tsieineaidd a'r ffaith bod maint y boblogaeth yn gymharol fach.

Nid oedd treialon gyda rhwydi trapio pysgod *fyke* a gynhaliwyd yn 2013 a 2014 yn llwyddiannus mewn dal y crancod yn yr ardaloedd oedd yn cael eu profi. Gallai bod sawl rheswm am hyn gan gynnwys y math o gyfarpar a ddefnyddiwyd ar gyfer y rhywogaeth targed, bod y lleoliadau yn amhriodol, yr amodau gwaith yn anffafriol neu maint samplu cyfyngedig mewn perthynas â maint cymharol fechan yn y nifer o grancod.

Roedd arolygon cranc manegog y draethlin (*shorleine mitten crab*) yn llwyddiannus mewn dod o hyd i'r cranc ifanc. Er hynny, byddai angen dull monitro llym a systematig er mwyn defnyddio'r fethodoleg hon i ganfod maint y poblogaethau neu'r newidiadau yn y poblogaethau. Mae'r fethodoleg yn gyfyngedig i ardaloedd llanw dalgylch y Ddyfrdwy.

Samplwyd ar gyfer y cranc mynegog Tsieineaidd bychan anaeddfed (*Chinese mitten crab megalopae*) mewn dau leoliad yn aber afon Dyfrdwy er mwyn gweld y newidiadau ym maint y boblogaeth. Fodd bynnag, ni chafodd unrhyw granc bychan anaeddfed ei ddarganfod yn yr ardaloedd a samplwyd. Mae hyn yn debygol o fod oherwydd nad oedd digon o leoliadau samplu ac na wnaethpwyd digon o ymdrech a bod diffyg digon o leoliadau samplu i ddangos y boblogaeth gyfredol yn hytrach nag absenoldeb llwyr o grancod ifanc anaeddfed yn yr aber. Cafodd rhywogaethau o gramenogion eraill eu darganfod yn llwyddiannus.

Cafodd nifer o gofnodwyr data eu lleoli mewn lleoliadau gwahanol ar hyd rhan isaf Afon Ddyfrdwy er mwyn monitro rhai amodau amgylcheddol (tymheredd a halwynedd) ar gyfer rhagfynegi presenoldeb y gwahanol gamau o hanes bywyd cranc mynegog Tsieineaidd. Mae canlyniadau lleoliad cofnodi data yn cadarnhau bod y crancod yn y Ddyfrdwy yn gorfod gorchfygu cored Caer er mwyn cwblhau eu cylchred bywyd. Gallai hyn ddarparu cyfleoedd posib ar gyfer rheoli'r rhywogaeth yn y dyfodol.

Mae'r cofnodion ar gyfer y cranc manegog o drap pysgod Cored Caer yn rhoi mesur o faint y boblogaeth berthnasol rhwng blynyddoedd ac fel y mae'r poblogaethau yn ymddangos i fod yn cynyddu'n raddol. Mae maint a phwysau'r cranc a'r amserau y maen nhw’n cael eu dal yn awgrymu bod y trap yn dal crancod hyn sy’n ymfudo i lawr yr afon yn bennaf. Mae rhai cofnodion yn dangos y rhai ifanc yn symud i fyny’r afon rhwng y gwanwyn a’r hydref. Fe all bod tymheredd oer y dŵr yn y gaeaf yn ffactor mewn cyfyngu ar allu’r larfa i oroesi yn yr aber. Mae hyn yn gallu arwain at amrywiadau ym maint y boblogaeth. Mae angen cofnodion mwy hirdymor i fesur yn union beth sy’n digwydd yn y safle hwn.

Dylai poblogaeth cranc manegog y Ddyfrdwy gael ei fonitro’n rheolaidd yn y dyfodol i asesu effeithiau ecolegol ac economaidd ac er mwyn datblygu cynlluniau rheoli. Fe allai canlyniadau dadansoddi DNA sy’n cael ei gwneud ar hyn o bryd roi gwell tystiolaeth i ni o sut orau i reoli a pharatoi canllawiau a pholisïau perthnasol ar gyfer y rhywogaeth.

Comisiynwyd yr adroddiad hwn cyn i’r rhywogaeth gael ei rhestru o dan reoliad newydd Rhywogaeth Oresgynnol Estron yr Undeb Ewropeaidd a’i nodi fel Achos o Bryder i’r Undeb.

2. Executive Summary

The Dee Chinese mitten crab project set out to investigate the population of Chinese mitten crab in the Dee catchment and achieve a number of objectives including awareness raising, trialling and evaluating potential methods to monitor and manage the population and make inferences about the current and future status of the species in the Dee catchment.

The awareness raising outreach work programme was successful in reaching a wide range of user groups in the Dee catchment including water abstraction utility companies, recreational and commercial fishermen and other river users, informing them of the issues and risks associated to the Chinese mitten crab. However, it is difficult to assess exactly how effective outreach such as this is in encouraging the reporting of species and preventing further spread. This may be related to the cryptic nature of the Chinese mitten crab and the fact that the population size is still relatively small.

Modified fyke net trapping trails conducted in 2013 and 2014 were not successful in capturing mitten crab in the locations tested. Several reasons may explain this including incorrect equipment type for the target species, inappropriate deployment locations, adverse working conditions or limited sampling effort in relation to the relatively low mitten crab population size.

Shoreline mitten crab surveys were successful at detecting juvenile mitten crab. However, a rigorous and systematic monitoring approach would be required in order to use this methodology to detect population size or population change. The methodology is limited to tidal areas of the Dee catchment.

Sampling for Chinese mitten crab megalopae was conducted at two locations in the Dee estuary in order to trial its use as a proxy for changes in population size. However, no mitten crab megalopae were detected in the locations sampled. This is likely due to insufficient sampling effort and limited sampling locations at current population levels, rather than absolute absence of megalopae in the estuary. Other decapod species were successfully detected.

A number of data loggers were deployed at various intervals along the lower section of the River Dee in order to monitor certain environmental conditions (temperature and salinity) for predicting presence of the different life-history stages of Chinese mitten crab. The results of the data logger deployment confirms that mitten crabs in the Dee must overtop Chester weir in order to complete their life cycle which may provide potential opportunities for future management and control of the species in the future.

Mitten crab records from the Chester weir fish trap provide a measure of relative population size between years in which the population appears to be gradually expanding. The size and weight of mitten crab and timing of capture suggest that the trap is predominantly capturing adult crabs migrating downstream, with occasional records of upward migrating juveniles from spring to autumn. Cold winter temperatures may play a role in limiting larval survival in the estuary resulting in fluctuations in population size although a longer term dataset is required in order to ascertain the full implications at this site.

The Dee mitten crab population should be closely monitored in future years to assess potential ecological and economic impacts and develop population management plans. The results of DNA analysis currently being undertaken may provide insights into pathway management and help to develop appropriate guidance and policies for this species.

This report was commissioned prior to the species being listed under the new EU Invasive Alien Species regulation as a species of Union Concern.

3. Introduction

The Chinese mitten crab monitoring project was funded under the wider marine pathways project, a collaborative project undertaken by several organisations* within the UK and Ireland in order to contribute to the delivery of the Non-Indigenous species descriptor of the Marine Strategy Framework Directive (MSFD). The overall aim of the project is to protect marine biodiversity in the UK and Ireland by managing key pathways by which marine invasive non-native species (NNS) are introduced and spread. The project was funded until April 2015.

3.1. Background

The Chinese mitten crab (*Eriocheir sinensis*) is registered by the Invasive Species Specialist Group (ISSG) of the International Union for Conservation of Nature (IUCN) as one of the top 100 invasive species worldwide (ISSG, 2009). It is endemic to Asia, naturally occurring in rivers terminating on the east coast of China between the North Korean border and Hong Kong (Hymanson *et al*., 1999) (Figure1.). In addition to wild populations there is also extensive commercial aquaculture of *E. sinensis* in this region. Mitten Crabs have been introduced into Vietnam, extending their range into Southern Asia.

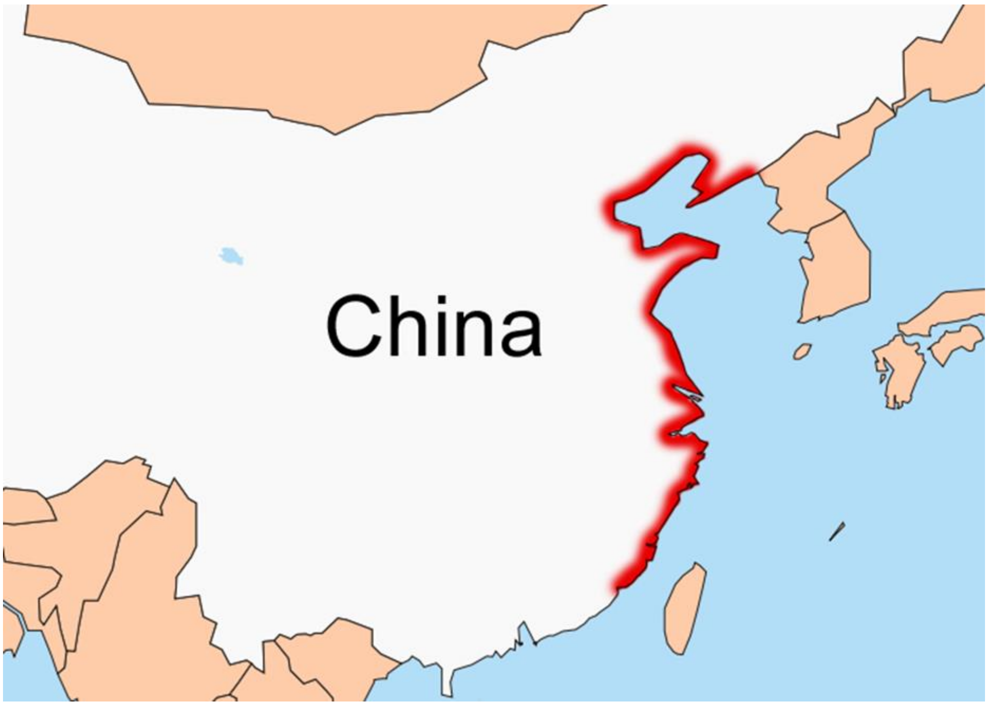

Figure 1. Chinese shoreline inhabited by native mitten crab populations (highlighted in red) extending from the North Korean border in the north, to Hong Kong in the south.
Source: Original map from www.freeworldmaps.net.

E. sinensis was first documented in Europe in 1912 in the River Weser, Germany. They were subsequently recorded dispersing through the canals and waterways across Europe. By the 1940's, populations of *E. sinensis* were recorded further across Europe in Denmark, Sweden, Finland, Poland, Holland, Belgium and France (Panning, A. 1939). Until the late 20th century, there were only two isolated reports of mitten crabs in the U.K; the River Humber in 1949 and the Thames in 1935.

The Chinese mitten crab was first recorded on the Thames in a water intake screen at the Lots Road power station, Chelsea in1935 (Clark *et al*., 1998). No further records were detected until 1973, after which the population became much more established (Herborg *et al*., 2005).

Numbers of mitten crabs recorded from the Thames remained relatively stable until 1992 when a dramatic increase in the number of records was observed. Following 1992 the Thames mitten crab population size and geographical extent continued to increase (Clark *et al*., 1998). The Thames population was primarily monitored through records of mitten crabs trapped in the intake screens of water abstraction facilities. However, recently, other monitoring methods such as fyke netting and intertidal shoreline searches have been used with some success (Clark et al., 2008, Gilbey *et al*., 2008). These techniques form the basis of monitoring work trialled on the River Dee during this project.

3.2. Chinese Mitten Crab Biology

Mitten crabs are a catadromous species, migrating between fresh water and marine environments to reproduce. Each mitten crab life history stage requires specific environmental conditions which are summarised in Table 1. They spend the majority of their adult life in fresh water habitats, where they mature to reproductive size. Depending on mitten crab population density and habitat suitability, juvenile mitten crabs will migrate large distances up river to mature. Mitten crabs have been recorded up to 1400 km inland on the Yangtze River, China (Cohen & Weinstein, 2001).

Table 1. Salinity and temperature ranges reported for mitten crab life history stages
(Larval development values from Anger 1991, all other data cited from Cohen and Weinstein 2001)

Life stage	Salinity (psu)		Temperature (˚C)	
	Possible Range	Optimal Range	Possible Range	Optimal Range
Mating	5 to 31	10 to 17	No data	14 to 15
Embryonic development	3 to 33	15 to 20	No data	15 to 20
Hatching		≈23		15 to 20
Larval Development			12 to 18≤	15 to 18≤
to Zoea 1	10 to 32	15 to 25		
Zoea 1 to 2	15 to 32	25 to 32		
Zoea 2 to 3	15 to 32	20 to 32		
Zoea 3 to 4	15 to 32	15 to 32		
Zoea 4 to 5	15 to 32	25 to 32		
Zoea 5 to Megalopa	20 to 32	25		
Metamorphosis to Juvenile and settlement	10 to 32	25 to 32		
Juvenile and Adult growth		0	4 to 32	15 to 30

Depending on geographic location, mitten crabs take between one and five years to reach reproductive maturity. Once mature, they migrate downstream during late summer and autumn with peak migration typically in September or October. The timing of this seasonal migration is relatively consistent across all known populations although the specific cues remains unclear. Change in water temperature, increased rainfall (Cohen & Weinstein, 2001), photoperiod, and the lunar cycle (Rudnick, *et al*., 2005; Morrit *et al*., 2013) have all been suggested as potential cues for individual populations but, as yet, no study has identified a consistent cue across all populations.

Mating takes place in brackish conditions in the upper and middle estuary. Female mitten crabs then spawn and retain the eggs until the larvae hatch. Mitten crabs develop through five larval stages and a megalopa stage, all of which are planktonic. The first larval stage and the megalopa stage are euryhaline, tolerating brackish to marine salinities, however the intermediate stages require more saline conditions. Megalopae then undergo metamorphosis to become juvenile crabs and settle out of the estuary water column before migrating back up river to suitable habitat.

E. sinensis' optimal habitat is slow moving, warm and shallow water, with long flushing into large estuaries such as the River Dee. Adults are often found in water temperatures of 7-31ºC, but can tolerate periods of extreme cold. They have been found in the Hai River down to a water temperature of 0ºC in China and established populations are present on North Sea coasts where winter water temperatures can drop to 3-5ºC. However, it has been documented that larval development temperatures need to be greater than 12ºC with a salinity of at least 15 psu (Anger, 1991).

Juvenile and adult mitten crabs take refuge amongst vegetation, under rocks and boulders and are also known to create burrows in river banks, often in high densities (Figure 2). In the River Elbe, Germany, in the 1930's up to 30 holes were recorded per m^2. Burrows are generally found at intertidal areas, where river banks slope steeply and are made up of fine sediment. Burrow systems can be deep and complex and have led to concerns about river bank integrity and erosion risk resulting from high concentration of burrows (Panning, 1939).

Figure 2. An example of a high density of burrows in a river bank at Syon Bank flood meadow, London.
Source: www.nhm.ac.uk

Chinese mitten crabs are broadly omnivorous, however the composition of their diet changes throughout their life history. The planktonic larval stages mainly consume phytoplankton and other zooplankton. After settling out of the water column, the juvenile crabs are predominantly herbivorous, feeding on aquatic vegetation for their first few months. As they mature their diet becomes increasingly omnivorous, becoming opportunistic predators of other macroinvertebrates (Hymanson *et al*., 1999). They have also been reported to feed on dead fish and fish trapped in nets. More mature crabs are also known to forage out of water on river banks (Hymanson *et al*., 1999).

The Chinese mitten crab has a square shaped carapace, about 50-70mm in diameter, with four lateral spines and small notch between the eyes (Figure 3). The most prominent characteristic, however, is the dense brown setae (fur) found on the claw, forming ‘mittens’ (Figure 3).

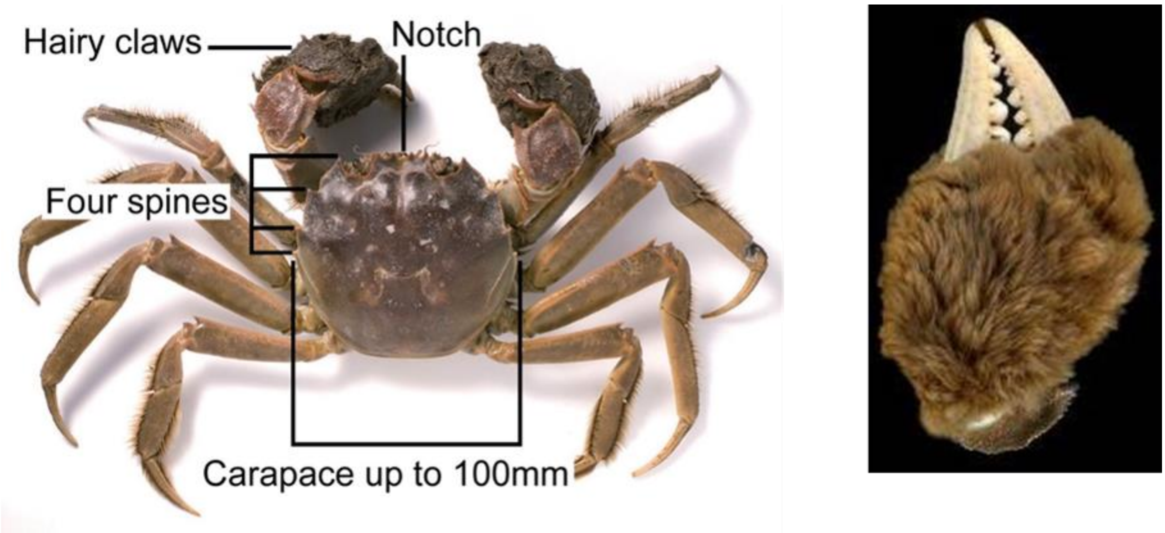

Figure 3. Left: A Chinese mitten crab with identifying features indicated. Right: Chinese mitten crab claw and setae.

Figure 4. Distinguishing morphological difference between male (left) and female (right)

They are a darker colour to Britain’s native shore crab (*Carcinus maenas*), being grey-green to dark brown. There is no distinct size difference reported between sexes. The only apparent visible difference between genders is on the underside of the crab. Females can be identified by the large abdominal flaps that extend to the edge of the abdomen when fully mature, whereas males have a V-shaped abdomen with narrow abdominal flaps (Rudnik *et al*., 2000) as shown in Figure 4. It is important to note that juveniles with a diameter <25mm, may lack conspicuous setae on the claws.

3.3. Introduction pathways

An invasive non-native species is any non-native plant or animal that has the ability to spread causing damage to the environment, the economy, our health or the way we live (CBD, 2009). It is now widely accepted that the spread of the Chinese mitten crab worldwide was a result of human-mediated activities and not a result of natural causes (Cohen and Carlton, 1997). A number of pathways (intentional and unintentional) have been identified that help to explain the transfer of this species around the world. These include:

- dispersal of larvae by currents
- passive dispersal of adults or juveniles on floating material
- transport of adults or juveniles by ship fouling
- transport of adults or juveniles in cargo
- transport of adults or juveniles on semi-submersible drilling platforms,
- barges and other long-distance slow-moving vessels
- transport of larvae or juveniles in ballast water
- transport of adults or juveniles in fisheries products
- transport of larvae in water with shipments of live fish
- escape or release from research, public, or private aquaria
- intentional transfer to develop a food resource

Figure 5. Ballast water being pumped from a large vessel.
Source: massbay.mit.edu

The most likely vectors for the Dee population are the discharge of ballast water (Figure 5) from European shipping and/or transport of adults or juveniles on slow-moving vessels and possibly the intentional transfer in order to develop a food resource.

Unintentional introduction and distribution may also result through fishing practices (Hymanson *et al*., 1999). Fishing vessels travel to various parts of the UK often using the same equipment, with little or no biosecurity measures in place, so may allow the accidental transportation of juvenile mitten crabs. Mitten crabs are able to survive for long periods out of water and so are particularly tolerant if caught up in nets. Another pathway to be considered is through importation for human consumption. Cohan & Carlton (1997) discuss how in Asia, the mitten crab is seen as a delicacy prized for its flavour, reporting that transportation of live crabs is known to occur in the US. Live mitten crabs are known to be available for consumption within the UK, and are currently sourced from suppliers in the Netherlands (King, 2013).

The practice of 'Life Release' a Buddhist tradition of saving lives of animals that are destined to be killed is also one possible explanation for the potential spread of mitten crab in the UK (*pers comms* Ben Wray). The practise is performed by most schools of Buddhism and particularly pertinent when considering mitten crab. Mitten crabs are seen as a delicacy and it is the developing ovaries of the females that are most valued. Once mature however, the females are much less sought after and it is therefore feasible that such animals could be released back into the wild as part of this ritual. Although the live release of mitten crab in the UK has never been documented, a similar event involving the release of Canadian lobster and Dungeness crab was reported off the coast of Brighton in June 2015.

3.4. Potential impacts

Colonisation of UK waters by *E. sinensis* has associated negative impacts both economically and ecologically. Concerns have been raised by the fishing industry (Ingle, 1986) as dragnet fisheries have found mitten crabs caught as bycatch have damaged the target species, reducing their market value. Fresh water fisheries also report crabs preying on trapped fish and damaging nets. Recreational anglers claim that colonisation by mitten crabs reduces the extent of fishing grounds during migration. Some industrial damage has also been associated with mitten crabs for example resulting from the presence of crabs in the intake screens of water abstraction facilities in water works and power stations. During the migration periods crabs have been reported to have caused blockage and clogging to equipment, resulting in reduced water flow and overheating. Manual removal of crabs and back flushing are lengthy processes which cause economic loss.

The ecology and ecological impact of *E. sinensis* remains poorly understood. In terms of predation, they are known to consume a variety of invertebrate prey and to compete with native species in a number of ecological niches. They are also known to consume buried Salmonid eggs and larvae and so an increase in crab numbers may affect population size in later generations for Salmonid species. It seems likely that their arrival would bring change to the community structure of native ecosystems through predation and competition. An indication of the potential ecological impact of mitten crab is provided by studying the research of other aquatic invasive species in the UK, such as native white clawed crayfish (Veldhuizen & Stanish, 1999). However, further research into the mitten crab specifically is required to fully understand the potential ecological implications.

3.5. Mitten Crabs on the River Dee

The first record of *E. sinensis* in North Wales was made in the River Dee in 2006. The Dee's source lies within the Snowdonia mountain range and stretches over 113km with a catchment area of 1816km^2 (Figure 6). It is a Special Area of Conservation (SAC), important for species such as Atlantic salmon *Salmo salar* (which may be directly threatened by *E. sinensis*), Otter *Lutra lutra*, River lamprey *Lampetra fluviatilis* and Sea lamprey *Petromyzon marinus* (JNCC, 2015). European eel *Anguilla anguilla* are also present. The River Dee is currently the only river situated in North Wales where invasion by *E. sinensis* has been confirmed. A substantial population is thought to be present on the River Dee, but this is largely based on anecdotal evidence. However, the Natural Resources Wales (NRW) operated fish trap located at Chester Weir has captured a total of 234 mitten crabs since 2007, 94% of which were caught in the months of September and October.

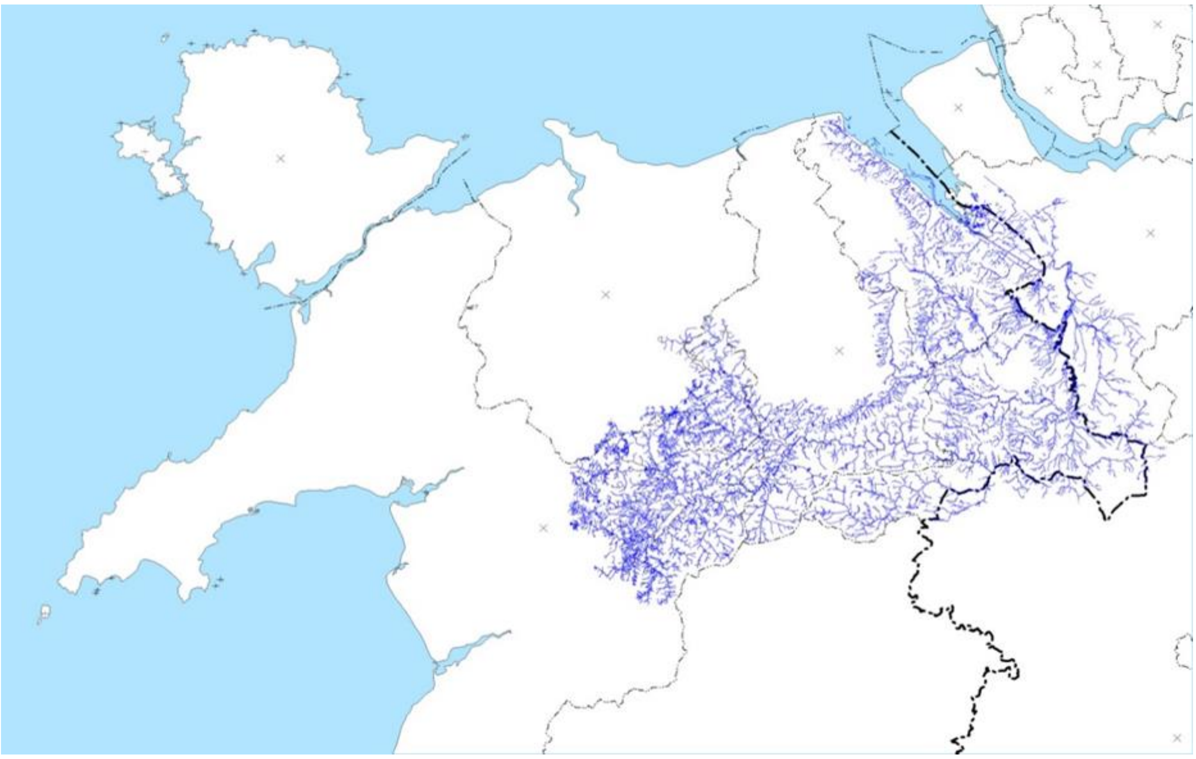

Figure 6 The River Dee catchment (dark blue).

3.6. Suitability of the Dee for Chinese mitten crab

Hymanson *et al*., (1999) define ideal mitten crab habitat as comprising "*a long, fresh water drainage with warm, slow moving water, and a large estuary*", with submerged vegetation. The lower catchment of the Dee conforms well to this description.

The Dee estuary is the sixth largest in the UK, with an area of 161km^2. The estuary is shallow with 90% of its area above low water on spring tides. The intertidal zone comprises dynamic sandbanks towards the estuary mouth, giving way to mudflats and salt marsh towards the more sheltered estuary head. There is a net movement of sediment into the estuary from both the river and Liverpool Bay due to stronger flood currents than the ebb currents, long shore drift and prevailing winds (Natural England & CCW, 2010). These processes may also be beneficial for the retention of planktonic mitten crab larvae within the estuary.

Immediately upriver of the estuary head is an 8km canalised section between Connah's Quay and Saltney, with a mixture of mud and sand substrate and little vegetation below the high tide water mark. As juvenile mitten crabs are largely herbivorous this section of the river does not appear to be favourable habitat. However, the remaining tidal section between Saltney and Chester weir is less heavily modified with more vegetation on the banks and intertidal zone.

Chester weir represents the tidal limit of the Dee with only spring tides above 9.1m overtopping it (King, 2013). Consequently, it is thought that the weir represents the threshold above which the river remains fresh. As mitten crabs develop to adults in fresh water environments they may have to pass upriver of the weir to complete their life cycle. Upstream of Chester, the Dee appears favourable to adult mitten crabs as the river meanders through a large floodplain extending south to the English/Welsh border at Erbistock. Throughout the floodplain the river is relatively slow moving with vegetated banks and submerged vegetation.

4. Project Aims

The aims of this project focussed on:

- Raising awareness of Chinese mitten crab in the Dee catchment;
- Trialling and evaluating methodologies to monitor and potentially control the Dee mitten crab population and,
- Using the data produced, make inferences about the population size and trends of Chinese mitten crab in the Dee catchment.

This report is divided into three sections based on the three main project aims each containing all elements of the work carried out.

5. Raising Awareness of Chinese mitten crab in the Dee catchment

5.1. Raising awareness objectives

An important element of this project was to raise awareness among stakeholders and the general public in the Dee catchment area about the Chinese mitten crab and the potential impacts. In addition to educating the public about this species, the project also set out to encourage the reporting of any Chinese mitten crab sightings to the national Chinese Mitten Crab Recording Project (CMCRP) (www.mittencrabs.org.uk)

Objective: Raise awareness of Chinese mitten crabs on the Dee and promote reporting of sightings to the mitten crab recording project.

Raising awareness of the Chinese mitten crab, and other invasive species in general, has the extra benefit of increasing understanding of the need for more stringent biosecurity practices among river users and therefore reduces the likelihood of human facilitated spread to other river catchments. It also informs the public about the work being undertaken to monitor and control Chinese mitten crabs as part of this project.

Objective: Contact organisations abstracting water from the Dee to determine whether mitten crabs can be monitored using records from intake filters.

On the river Thames the most comprehensive data on numbers of Chinese mitten crabs has come from records of crabs cleared from water abstraction intake screens. This project aimed to establish contact with companies abstracting water from the Dee to determine whether they encounter Chinese mitten crabs and whether it is possible to use this information to monitor the population.

5.2. Methodology used

The outreach work programme was targeted towards groups that use the river commercially, recreationally, or that are involved in river management. These groups were specifically selected as they were mostly likely to encounter or be affected by the presence of Chinese mitten crabs in the Dee. They included angling clubs, commercial fisheries or industrial operators such as those abstracting water from the Dee. In addition more generalised awareness raising activities were targeted towards other stakeholders including conservation organisations, local authorities and local communities.

Talks and presentations
Project staff delivered presentations on mitten crab awareness to a range of river stakeholders and interested groups. These took the format of both specific meetings organized to discuss Chinese mitten crabs, or as part of wider initiatives such as the Dee Invasive Non Native Species Project (organised by the North Wales Wildlife Trust). Presentations covered general biology, identification, life history, distribution of mitten crabs, potential threats and impacts (e.g. economic and ecological), and invasion pathways, such as ballast water, fisheries and intentional introduction. They also described and explained the aims, methods used, and current results of this project. Furthermore, these presentations acted to promote the nationwide Mitten Crab Recording Project and provided information about how and where to report sightings.

Media campaign
A wider awareness raising campaign involved use of local and regional media: through radio interviews with BBC Radio Wales and a newspaper article in the Evening leader. Furthermore, a bi-lingual campaign poster, aimed at identifying the specific characteristics of the mitten crab, drawing attention to the potential threats and where to report any sightings (Figure 7 & Appendix 10.1), was displayed at locations frequented by river users and local communities such as Yvonne's café at Connah's Quay docks, targeting the local angling community and fishermen operating from Connah's Quay. Small identification cards containing similar information were produced and distributed with the poster, at the presentations, and to interested members of the public encountered by staff undertaking other monitoring activities.

Figure 7. Chinese Mitten crab campaign poster.

The poster was distributed in public places frequented by river users.

Volunteer participation in monitoring

In addition to presentations and distribution of printed media, efforts were made to actively involve the public with Chinese mitten crab monitoring through volunteer events. Juvenile search days were organised at sites along the River Dee. Universities and colleges were contacted in order to promote these voluntary positions to students. Voluntary positions were advertised through the Wildlife Trusts' Facebook page, to help attract an audience for voluntary positions.

In 2013, all the work undertaken on assessing sampling techniques was achieved through the use of volunteers. The boat work was undertaken with a volunteer who took a lead on this part of the project in 2013. Volunteers also assisted during 2014 with fyke netting, megalopa sample collection and data retrieval from the data loggers.

Investigation of water abstraction operations for mitten crab monitoring

An objective of the study was to determine the feasibility of obtaining records of Chinese mitten crab from industrial operations and utilities companies involved in abstracting water from the Dee. Blockage of water abstraction points by migrating crabs has been identified as a potential impact of invading mitten crab populations. Furthermore, data on numbers of mitten crabs cleared from water abstraction intakes was the main method used to monitor the Thames population until the 1990s (Clark *et al*., 2008). In the light of this, it was proposed that certain operations on the Dee may routinely collect information/data on Chinese mitten crab relevant to this study. Contact was made with the seven organisations that abstract the largest volumes of water from the lower Dee to ascertain whether:

- These organisations had encountered Chinese mitten crabs in the course of their operations.
- Their water abstraction technology was suitable for monitoring Chinese mitten crab numbers.
- This, and future projects could work with these organisations to monitor Chinese mitten crabs on the River Dee.

5.3. Results and discussion

Public Engagement and Outreach
The outreach activity from this project can be evaluated in terms of: the range of audiences reached; the extent to which levels of volunteer engagement were increased; the extent to which outreach activity might account for any increase in recorded sightings; and the outcome of investigation to determine the potential for working with water abstraction companies to monitor mitten crabs on the Dee.

Range of Audiences Reached
The outreach work programme was successful in reaching most groups targeted. This is evidenced by on-going relationships developed with commercial, recreational and river management organisations and increased volunteer engagement. See Table 2 for details of stakeholder engagement events.

The presentations delivered appear to have been particularly effective in engaging the ongoing involvement of stakeholder organisations, for example some commercial organisations and utilities companies involved in water abstraction requested further training. Following a presentation, the Conservation Officer from one angling club requested resources including a preserved specimen and printed media so that he could discuss Chinese mitten crabs when attending a number of angling events and country shows. This individual continues to be in contact and has reported high levels of interest.

Table 2. Summary of presentations delivered to stakeholders in 2014

Audience	Date	Presentation	Number of Attendees
NWWT Wrexham branch	Spring 2014	Dee INNS project presentation	25
Biosecurity seminar, Llangollen	16th April 2014	Dee INNS project presentation	15
United Utilities, Huntington	19th June 2014	General Presenation on Chinese Mitten Crabs	20
Prince Albert Angling Society	7th August 2014	General Presenation on Chinese Mitten Crabs	50
NWWT Conservation Committee Mold	17th November 2014	Dee INNS project presentation	10
Water Framework Directive stakeholders Llandrindod Wells	18th Nov 2014	Seminar and Workshops	30+
Dee INNS Project stakeholders Acrefair	18th Nov 2014	Dee INNS Project annual meeting	

Media coverage was used as a means of addressing wider audiences and raising awareness with local communities, however the effectiveness of this approach is difficult to quantify. Project officers were interviewed by BBC Radio Wales programmes on 13th July and 16th of September 2014. Both sets of interviews were used within conservation and science themed programmes which were broadcasted to audiences across Wales. Additionally an illustrated feature article was published in the Evening Leader newspaper which is distributed across North Wales. Although it is probable that this media coverage effectively increased awareness across wider Welsh populations this is difficult to assess. Feedback was received in response to this coverage, although as this was most often provided by people who the project officers came into contact with in other contexts, such as when working with volunteers.

Printed media, such as mitten crab identification cards and information leaflets, were distributed to a number of venues within the area including country parks, outlets selling angling equipment, visitor attractions and tourist information centres. These materials were also handed out at events for volunteers and the general public run by project officers, and at regional events and county shows (Table 3).

Table 3. Key events at which printed material was distributed

Event	Date	Number of Attendees
Big Dee Day – The Invasion launch event	27th/28th June	50
Denbigh and Flint Show	21st August 2014	2-3,000
Merioneth County Show	27th August 2014	2-3,000
Llandegla Village Fete	6th September 2014	500-1,000

It is probable that distribution of printed media as well as public contact with project officers will have raised awareness, but it is not possible to quantify the direct result. Again there is some anecdotal evidence of increased awareness and at least one mitten crab sighting has been reported by an individual who had seen these materials.

Volunteer Engagement

A number of volunteer engagement events were run by project officers with the aim of raising awareness, increasing participation, and contributing supplementary data to monitoring of mitten crab populations. These included days involving volunteers in surveys of juvenile mitten crab populations. Additionally volunteers involved in both 'Big Dee Day' events on 6th of June and 19th of September 2014, took part in awareness raising activities related to issues concerned with invasive species, including mitten crabs.

These volunteer days also teamed up with the Marine Alien Species Champions Project, where 'Champions' attended the volunteer juvenile Mitten crab search days with the aim of spreading the word about the project across and number of organisations and sectors. The project was run by the North Wales Wildlife Trust (NWWT) and grant funded by NRW under the Marine Pathways Project. For more information and report see http://www.nonnativespecies.org/index.cfm?sectionid=105
The juvenile search events were not well attended. There was a limited response to the advertising of these events and a higher than expected dropout rate after volunteers had signed up. There may be several reasons for this: juvenile searches involved a greater time commitment and physical activity than other volunteer activities, these sessions were planned for week days when many working age participants would have been unavailable, and these volunteer sessions may not have been advertised appropriately to reach individuals who may have wanted to take part. Volunteer activities are routinely advertised on the NWWT websites and in general the profile of volunteers may include elderly or retired people, or family groups who may not have felt able to engage in the juvenile search day activities. If volunteers were to be recruited for similar activities in the future, an alternative

approach would be needed. For example, recruitment and attendance may have been more successful had these events been advertised to different groups, or taken place on weekends when a greater number of volunteers would have been available.

Recording of Mitten Crab Sightings

A key objective of the project was to raise awareness among stakeholders and the wider public in order to generate increased data as a result of possible Mitten crab sightings on the Dee. It was predicted that if a greater number people were aware of the presence and potential issues associated with mitten crabs, then any potential sightings would result in an increase in reporting.

However, results on the Mitten crab recording project website indicate that this has not been the case in that no additional mitten crab sightings were reported for the Dee during 2013 to 2014. This suggests that the promotion of the recording project was unsuccessful, however, there may be other reasons for a lack of additional records.

Mitten crabs are a relatively cryptic species, particularly in the non-tidal river (upstream of the weir), and the population is still relatively small. As shown from the targeted monitoring efforts the capture rate of crabs is low. The few sightings reported to this project and the cryptic nature of species may instead suggest that the public and stakeholders simply are not encountering mitten crabs at present.

A further factor may be that when sightings have occurred they have not been formally reported. Anecdotally, three reports of mitten crab sightings were provided directly to the project team from engaged stakeholders. On the 5th September 2014 an Environment Agency Biodiversity Officer contacted project staff to report 25 Chinese mitten crabs found by river bailiffs in a boat near Almere (SJ 405 563). It was presumed that these crabs were abandoned by-catch from illegal eel fishing. Later, in December 2014, anglers from Rosset and Gresford Fly Fishers contacted project staff to report the sighting of a Chinese mitten crab on the River Alyn, a tributary of the Dee, in Rosset (SJ 383 563). Finally, a commercial fisherman contacted project staff in December 2014 to report catching approximately eight adult sized mitten crabs per tide in the estuary from mid-November.

In addition to reporting of sightings, it is also possible that increased awareness among river users, during the course of this project, may have promoted additional biosecurity, thus limiting the risk of accidental human introductions to other nearby rivers. If this is the case it demonstrates the value of maintaining levels of awareness among stakeholders. Continued promotional work may still be beneficial in the future as it could identify any increase in the population size.

Water abstraction operations and their use to monitor mitten crabs on the Dee

Liaison work was carried out with different industries on the Dee involved in water abstraction (Table 4) to determine whether they encounter Chinese mitten crabs during their operations, and whether this data could be used to monitor the population. The following companies were contacted: Deeside Power, Dee Valley Group, Dwr Cymru, EON, TATA steel, and United Utilities.

Table 4. Outcomes from initial contact in January 2014 with utilities and water companies

Organization	Operational Activity	Outcome
Deeside Power	Power Generation	Site visited Data from jetty monitoring of biodiversity and water quality provided Mitten crab cards distributed to staff members
Dee Valley Group	Water Utility	Confirmed that they abstract water from the Dee but no records
Dwr Cymru	Water Utility	Information on project provided No further response
EON	Power Generation	Information on project provided No further response
Dwr Cymru	Water Utility	Information on project provided No further response
TATA steel	Steel Production	Agreed to possible setting of monitoring nets from their jetty Interest in further training in relation to mitten crabs
United Utilities	Power Generation	Two site visits Agreed to location of fyke nets downstream of the weir. Requested staff training/presentation – delivered June 2014

None of these organizations reported any sightings of Chinese mitten crabs and none had identified mitten crabs as presenting a potential risk to their operations. Records from abstraction operations do not appear to be a viable data source for mitten crab monitoring on the Dee. Partly this is due to use of modern abstraction technology, which provides automated blockage prevention and does not use filter screens. Consequently manual removal of blockages is not required, leading to low likelihood of encountering mitten crabs by staff. In general, there seemed to be little potential in harnessing routine inspection within the existing operations of these companies to monitor mitten crab populations. Nevertheless relationships were formed with some of the organizations, predominantly Deeside Power, Tata Steel and United Utilities. These companies were particularly concerned to be vigilant against any potential risk to their operations that an increase in the mitten crab population might cause. This means that the potential for future collaboration in monitoring mitten crab populations may remain. In the meantime raised awareness of mitten crabs has been achieved within these organizations and should the species be encountered it is more likely that they will be reported.

6. Developing monitoring methods

6.1. Monitoring objectives

A further aim of the project was to develop, and assess the suitability of a range of techniques to monitor the population of Chinese Mitten Crabs in the River Dee.

Objective: Determine whether conducting shoreline surveys for juveniles can identify the presence of mitten crab.

It was proposed that manual searches of the river banks would be appropriate to detect and monitor the river Dee *E. sinensis* population (King, 2013). Juvenile mitten crabs have been shown to shelter under rocks above the waterline at low tide and similar intertidal shoreline surveys have been used on the Thames to investigate annual and seasonal variations in abundance (Gilbey, *et al*., 2008). A series of shoreline searches were conducted during summer and autumn 2014 to test this methodology.

Objective: Determine whether deployment of fyke nets positioned at multiple locations in the river can capture migrating adults.

Historical efforts to control this species in other rivers have typically been focussed on capture and removal of adult crabs. Fyke nets have been used successfully on the Thames to capture mitten crabs and monitor their population, and may have been effective in controlling the population on the Guadalquivir River in Spain (Garcia-de-Lomas *et al*., 2010). Furthermore, Clark *et al*. (2008) compared the effectiveness of fyke nets with baited crab pots traditionally used to capture native Decapod species and found fyke nets to be significantly more efficient. Fyke nets were trialled in both 2013 and 2014 using differing methods and deployment locations to determine a suitable monitoring methodology for the Dee.

Objective: Determine whether the incidental trapping of mitten crab, as by-catch at NRW’s Chester Weir fish trap, is suitable to detect and monitor their population in the River Dee.

Project staff collaborated with staff at NRW’s fish trap at Chester to collect data. The purpose of the trap is to monitor migrating salmon and sea trout, however numbers of mitten crab are trapped each year and the fish trap staff have kept records of the species dating back to 2007. Since these records cover a larger time span than this project, they were useful in assessing potential trends in population size and provided a point of comparison to measure the effectiveness of other trapping methodologies. Given the consistent success of trapping the species, analysis of the conditions making the fish trap favourable to mitten crabs would allow development and refinement of future adult trapping techniques.

Objective: Deploy megalopa collectors in saline environments of the Dee to establish whether mitten crab larvae were present.

If the Chinese mitten crabs in the Dee are able to reproduce in the estuary it is predicted that the numbers of megalopae and adult crabs found within the Dee river and estuary will continue to increase; therefore it is important to monitor changes in megalopae abundance. Megalopae and larval abundances have been suggested as a predictor of future adult population dynamics (Blumenshine *et al*., 2011). It was hypothesized that Chinese mitten crab megalopae are present in the River Dee estuary; however this has never been empirically tested. King (2013) suggested that surveys of larvae or megalopa in the river estuary could infer reproductive status of the mitten crab population on the Dee and may also provide a means to assess population size and trends (B. Wray 2014, *pers. comm*).

Objective: Data loggers to be placed in the River Dee to identify whether variations in the environment will limit spatial distributions of mitten crab at different life history stages.

Differences in salinity are thought to be a major factor in limiting the range of each life history stage of Chinese mitten crab within a river. Therefore the primary aim of the data loggers was to measure environmental conditions throughout the gradient from fresh to saline conditions, which define the ranges of each life history stage. In particular, the use of the data loggers aimed to determine whether the Chester weir acts as a barrier between fresh and saline conditions and therefore whether Chinese mitten crabs must pass the weir to complete their life cycle.

Secondly, the cues that drive adult downstream migration are currently unclear; however, changes in water temperature are one factor that has been suggested. Temperature data recorded using the data loggers may provide useful insights into potential environmental cues when combined with records from other monitoring techniques.

6.2. Monitoring Methodology

Juvenile Searches

Sites suitable for juvenile surveys must have a high density of refugia, such as rocks on the surface of the sediment, on the intertidal riverbank and be safely accessible on foot. There are currently three sites identified as suitable for riverbank juvenile searches; the bank immediately down river of Chester Weir fish trap (SJ 407 657) (Figure 8); the bank down river from Crane Wharf NRW site, adjacent to The Cop park (SJ 328 682); and Sandycroft (SJ 397 663) (Figure 9).

Juvenile mitten crabs have been recorded using refugia on the surface of sediment (Gilbey *et al*., 2008). Refugia which are submerged in the substrate, and which have no obvious opening to the underside, were not checked. Also, turning refugia resting in water disturbed the substrate, obscuring any potential juvenile crabs, so was avoided.

Figure 8. Chester Weir intertidal juvenile survey site location

Surveys were conducted at low tides when the greatest area of river bank is exposed and refugia are most accessible. The timing of surveys also allowed more time to conduct surveys before survey sites were inundated by the tide. The suitability of refugia uncovered by the receding tide may change over time after high tide. Time after high tide was recorded for each survey to control for any effect it may have had on *E. sinensis* juvenile abundance.

To quantify sampling effort total area surveyed was recorded. A GPS was used to plot the perimeter of the surveyed area from which the area (in m^2) was calculated. Alternatively, if a GPS was not available, the area was estimated by multiplying its width (from the lowest to highest point up the bank) by its length along the river. As the density of suitable refugia varied across survey sites, the number of refugia checked was also recorded. To avoid double checking refugia, each was marked once checked by scribing a line across it with chalk or in the silt on its surface (Figure 10). Times of survey start and finish were also recorded.

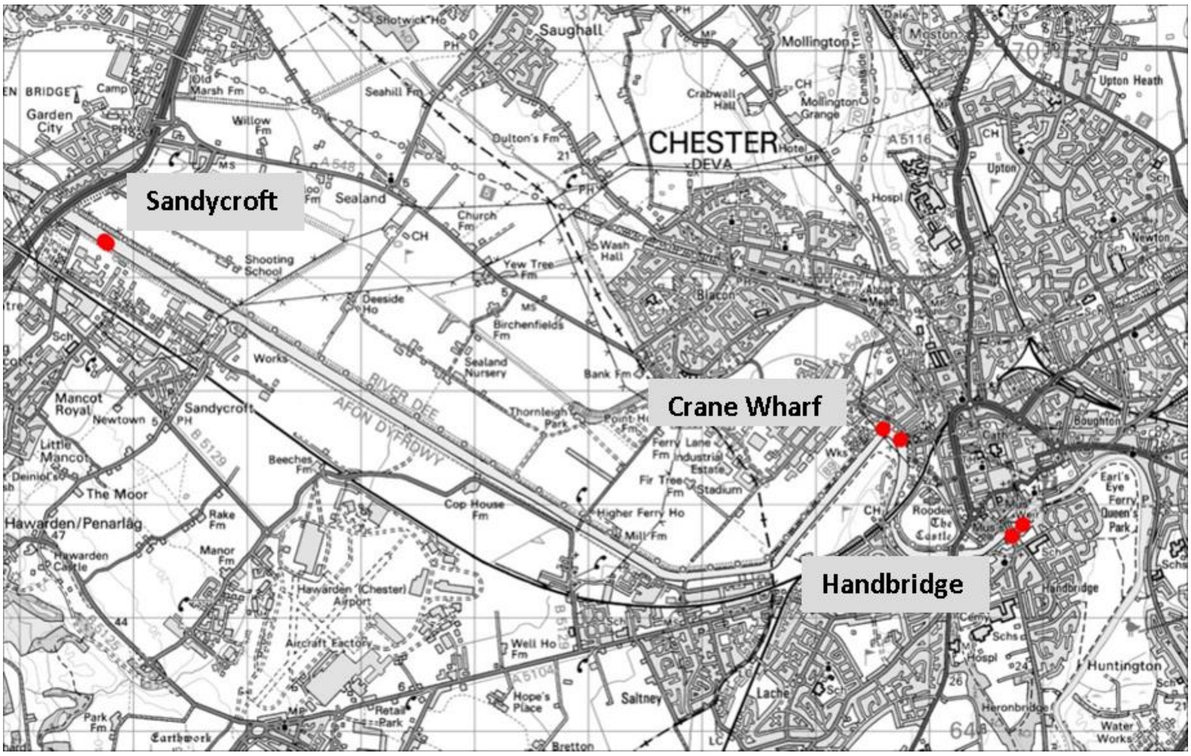

Figure 9. Shoreline survey locations (red dots): Sandycroft, Crane Wharf, Handbridge. Pairs of dots indicate up river and down river limits of each surveyed site.

Figure 10. Marking refugia (stone) using fingertip at a juvenile search at the Handbridge survey site.
Chester weir is visible in the background with the fish trap shown toward the top left of the image.

The species, sex (male/female/unknown), carapace width (mm), status (alive/dead), time found and any additional comments were recorded for every crab found. All crabs were placed into a container to prevent double sampling, and because it is illegal to release them (Figure 11). All native species were released immediately after the survey. *E. sinensis* were placed into a container with a secure lid for transportation from the site. Transportation was undertaken within relevant Transport of Live Animals Regulations. Mitten crabs were then killed by placing into a freezer. Once dead, weight was recorded. They were then stored for further analysis or disposed of in accordance with guidance from the Animal Health and Veterinary Laboratories agency.

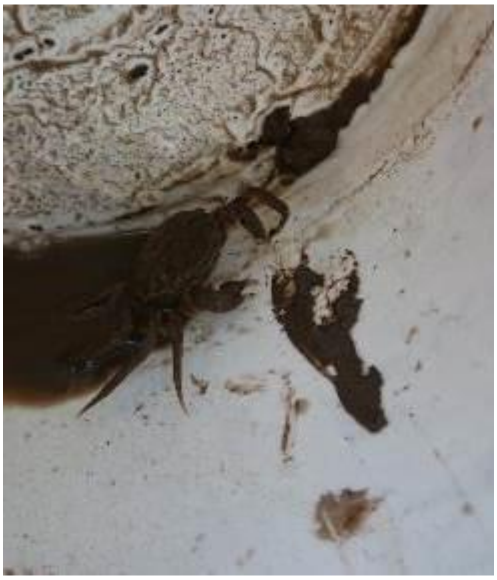

Figure 11. Juvenile mitten crab placed inside container from juvenile search

Fyke netting

A licence was obtained from the Environment Agency (England) which required liaison with Natural England and NRW to use the fyke nets for trapping on the Dee (Appendix 10.2). Due to the Dee's status as both a Special Area of Conservation and SSSI, a number of modifications were made to the fyke nets to reduce the risk of harm to protected species. These modifications are summarised in Table 5.

The trap consisted of an approximately 3.2m long conical net with a circular entrance 50cm in diameter (Figure 12). The net had an overall mesh size of 7-9mm with the exception of the end of the trap cone which had been adapted by replacing the existing mesh with a 40mm mesh to allow non-target species such as eels, lampreys and small fish to escape. The entrance of the trap cone was fitted with an otter guard to prevent otter from becoming entangled in the net and prevent larger fish, for instance migrating salmonids, from entering the trap. The more usual layout of a fyke net showing the six metre long leaders (wings) which would act to potentially funnel crabs into the trap can be seen in Figure 13, this confuguration was used in the 2013 trapping season.

Table 5. Feature list for the River Dee and Bala Lake/Afon Dyfrdwy a Llyn Tegid SAC and mitigation measures taken.

Species	Designation	Mitigation
Floating water-plantain (Luronium natans)	SAC - Annex II species – primary reason for selection of this site	N/A
Atlantic salmon (Salmo salar)	SAC - Annex II species – primary reason for selection of this site	Nets facing upstream - enabling capture of Chinese mitten crab moving downstream, and also avoid potentially capturing Salmon or Lamprey, nets will be monitored for ingress of non-target species with activity being ceased if mortality occurs.
Otter (Lutra lutra)	SAC - Annex II species present as a qualifying feature, but not primary reason for site selection	Installation of otter guard to net.
Sea lamprey (Petromyzon marinus)	SAC - Annex II species present as a qualifying feature, but not primary reason for site selection	Nets facing upstream, nets will be monitored for ingress of non-target species with activity being ceased if mortality occurs.
Brook lamprey (Lampetra planeri)	SAC - Annex II species present as a qualifying feature, but not primary reason for site selection	Nets facing upstream, nets will be monitored for ingress of non-target species with activity being ceased if mortality occurs.
River lamprey (Lampetra fluviatilis)	SAC - Annex II species present as a qualifying feature, but not primary reason for site selection	Nets facing upstream, nets will be monitored for ingress of non-target species with activity being ceased if mortality occurs.
European eel (Anguila anguila)	IUCN critically endangered, UK BAP, Section 42 Wales	Installation of 40mm gauge net at cod end of trap. This species known to survive long periods in net before being released.

Figure 12. Fyke net without leaders as used in the 2013 trapping period.
Otter guard visible attached in the entrance of the trap (left). 40mm mesh attached to end of fyke nets to reduce eel by-catch (right)

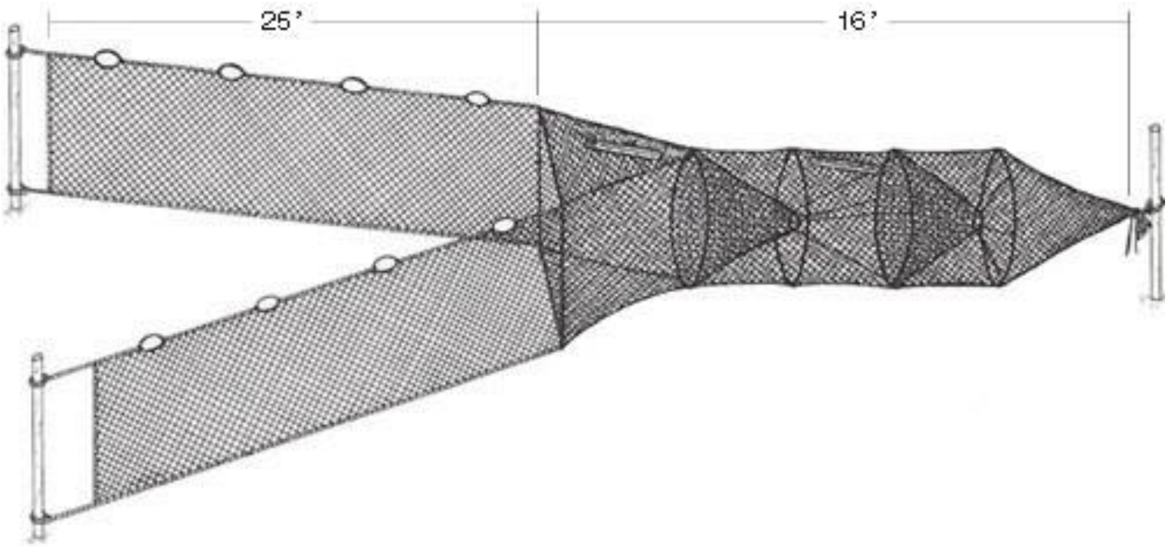

Figure 13. Layout of a typical fyke net, with leaders attached as used during the 2014 trapping period.

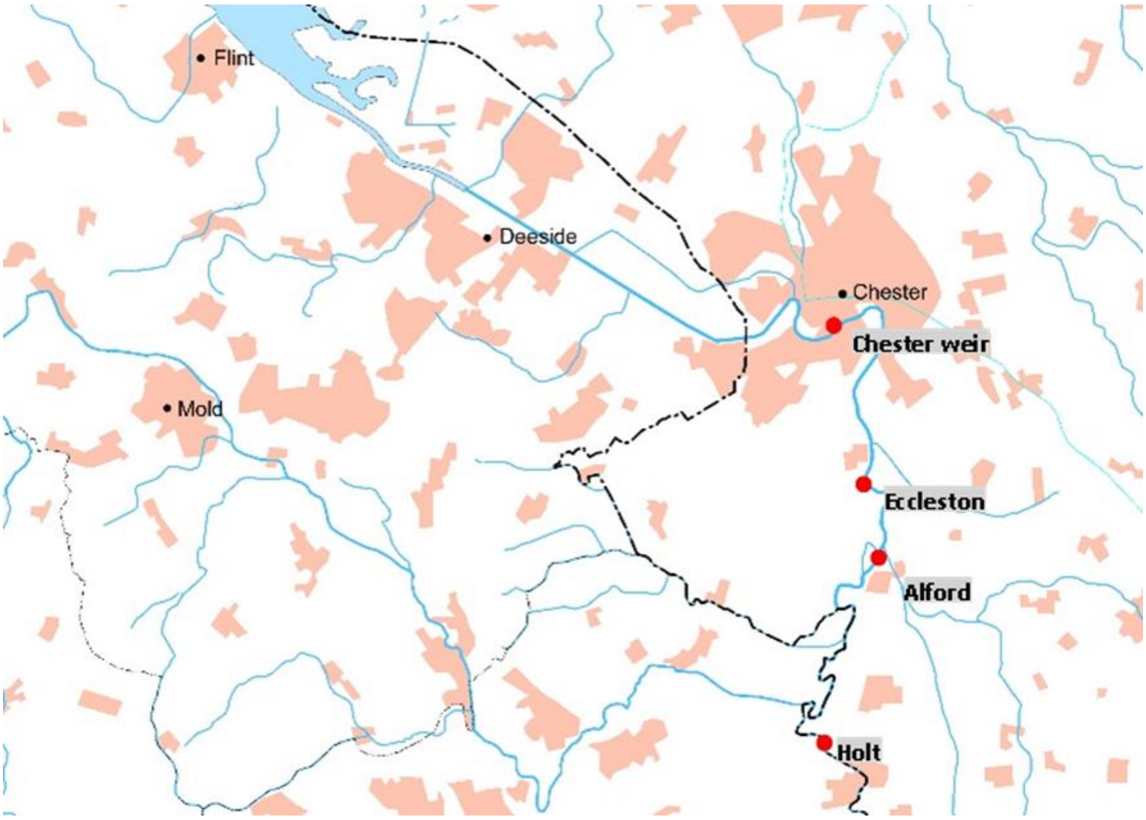

Figure 14. 2013 study area and location of fyke nets within the study area (illustrated using red dots).

Trapping Period 2013

Fyke nets were set at four different sites between the estuary and Holt, Wrexham: Chester Weir (SJ40673 66044), Eccleston (SJ41440 61945), Alford (SJ41833 60054), and Holt (SJ40432 55270) (Figure 14).

To ensure net security and avoid interference from the public, inaccessible sites were chosen away from public footpaths and where possible on private land where access had been granted. Baler twine was also used to attach the nets to the river bank to make the traps less conspicuous in areas where public access issues couldn't be avoided.

The nets were set for between 24-48 hours in accordance with the methodology as used to capture *E. sinensis* in the Thames River. A maximum of four nets were set at one time. One net was set permanently at Holt (checked every 24 hours), while the others were deployed on 8 separate occasions for a 24 hr period. Trapping took place between August and October 2013.The date and time the nets were set and retrieved, and the net contents were recorded (Table 7). All species caught in the trap and their status (alive/dead) were recorded.

All nets and equipment were soaked in Virkon after use and left to dry in the air following "Check- Clean- Dry" practice. To reduce the risk of spreading any invasive species. The traps were tagged with a licence number and always used at the same site.

Table 7. 2013 Fyke net deployments and outcomes.

Date Deployed	Date Retrieved	Soak time (hrs:mins)	Location	Catch	Comments
12/09/2013	13/09/2013	20:35	Chester weir	None	
12/09/2013	13/09/2013	19:15	Eccleston	None	
12/09/2013	13/09/2013	19:35	Alford	None	
17/09/2013	18/09/2013	25:20	Chester weir	1 Flounder (Platichthys flesus)	22cm
17/09/2013	18/09/2013	25:10	Eccleston	None	
17/09/2013	18/09/2013	25:05	Alford	None	
17/09/2013	N/A*	N/A*	Holt	None	*Set continuously until 11/2013 and checked daily. No catch.
26/09/2013	27/09/2013	25:20	Chester weir	None	
26/09/2013	27/09/2013	25:50	Eccleston	None	
26/09/2013	27/09/2013	25:40	Alford	None	
03/10/2013	04/10/2013	21:30	Chester weir	4 Flounder (Platichthys flesus)	13cm alive, 13cm dead, 12cm dead, 10cm dead
03/10/2013	04/10/2013	21:55	Eccleston	None	
03/10/2013	04/10/2013	21:50	Alford	None	
10/10/2013	11/10/2013	26:45	Chester weir	None	
10/10/2013	11/10/2013	26:40	Eccleston	None	
10/10/2013	11/10/2013	26:25	Alford	None	
18/10/2013	19/10/2013	18:55	Chester weir	None	
18/10/2013	19/10/2013	19:35	Eccleston	None	
18/10/2013	19/10/2013	19:40	Alford	None	

Trapping Period 2014

As part of the 2014 trapping period only one fyke net (with wings attached), was set downstream of the weir, in a stretch of river where it is assumed migrating adult *E. sinensis* must pass in order to enter the estuary environment. The net was set initially using a kayak and then a small inflatable boat at Crane Wharf (SJ39772 66587), a secure NRW site (Figure 15), therefore reducing the risk of interference from the public. The river is strongly tidal at this location and therefore setting the fyke net was only possible in the hour before low tide when flow rate is slowest and allowed the net to be set facing up river. For all sampling protocols see Appendix 10.2)

The inclusion of wings on the nets during the 2014 trapping period had the potential to increase the risk of by-catch of species of conservation importance. Therefore, the initial soak time of the net was limited to a period of 12-14 hours. By-catch of species of conservation importance was monitored closely to ensure that, when the net was retrieved, all of these species (if present) were alive and could be successfully released back into the river. Since no damage or mortality of species of conservation importance was evident, the soak time of the trap was increased up to a 48 hour period but checked at least every 24 hours. The trapping took place approximately once per week from August to late September. Further efforts were made to set the net throughout October but river conditions prevented successful deployment (Table 8).

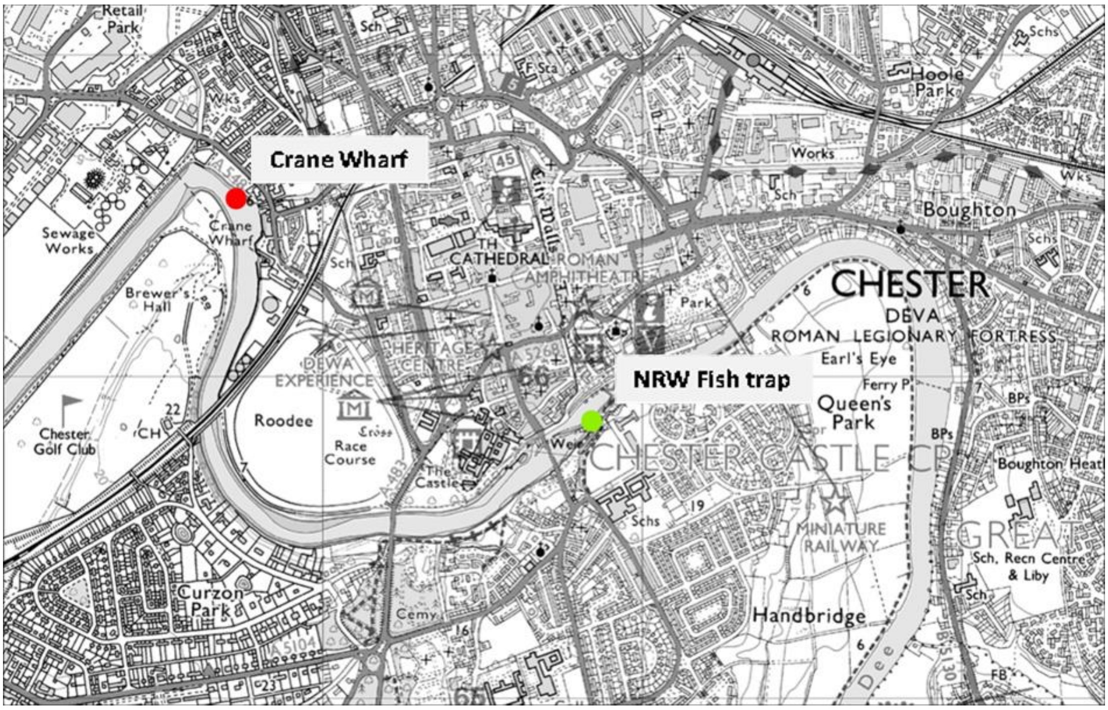

Figure 15. Crane Wharf 2014 fyke net deployment location (red) and NRW fish trap location (green).

Table 8. 2014 Fyke net deployments at the Crane Wharf site and outcomes.

Date Deployed	Date Retrieved	Soak time (hrs:mins)	Location	Catch	Comments
18/08/2014	19/08/2014	21:30	Crane Wharf	None	Eel (Anguilla anguila) in net which escaped during net retrieval
28/08/2014	29/08/2014	18:30	Crane Wharf	None	Net set approx. 15 minutes after high tide, the strong flow made setting difficult and unclear if net set properly. On retrieval net was twisted and partially inverted.
19/09/2014	20/09/2014	25:15	Crane Wharf	None	Net inverted and anchors dislodged, net reset
20/09/2014	21/09/2014	24:40	Crane Wharf	None	Still set correctly on retrieval however large tree branch caught in rope to bank
26/09/2014	27/09/2014	24:30	Crane Wharf	None	
17/10/2014	N/A	N/A	Crane Wharf	N/A	River flow too fast to set net at low tide
21/10/2014	N/A	N/A	Crane Wharf	N/A	River flow too fast to set net at low tide
24/10/2014	N/A	N/A	Crane Wharf	N/A	River flow too fast to set net at low tide

Access to and security of net deployment sites
In both 2013 and 2014 there was no evidence that nets had been tampered with by river users or the wider public indicating that the chosen deployment sites were suitably secure.

At sites used in 2013 nets were deployed directly from the riverbank. However, at Crane Wharf a boat or kayak was required; therefore safe access to the net deployment position was limited to periods when the river was slower moving. At low tide access to the river at Crane Wharf required navigating a steep muddy bank. Due care was taken and appropriate personal protective equipment was used. Aside from this, and constraints due to river conditions, the river was easily and safely accessible from Crane Wharf.

Fish Trap
Since 1991, Chester Weir fish trap has been used to tag and trap adult salmon and sea trout in order to estimate the total run of returning fish each year and to collect information on their biology. Use of this information has allowed for an improved understanding of factors effecting survival and abundance of these species (Dee Angler Report, 2013).

Figure 16. The fish ladder leading up to the fish trap situated at the side of the weir. Photo taken looking downstream under normal high tide conditions

Trapping is carried out throughout the year but not continuously. The trap is situated at the head of the tidal Dee, acting as a partial trap during normal tidal conditions (Figure 16). Under extreme spring tide conditions or during periods of high rainfall when river levels are high, fish are able to pass directly over the top of the weir, thus bypassing the trap.

E. sinensis have been detected each year at the fish trap since 2007 and their numbers recorded. Since these records currently provide the most consistent data on Chinese mitten crab in the Dee, this project collaborated with the NRW fish trap staff to obtain data on mitten crab numbers at the trap and specimens for analysis.

Figure 17. Upstream gate from inside the fish trap preventing fish from continuing upstream (top). Downstream entrance to the fish trap directly above the fish ladder (bottom).

The trap is comprised of upstream (exit) and downstream (entrance) gates at either end of the trap (Figure 17). When the trap is being fished the downstream funnel gate is open allowing fish (or mitten crabs) to enter the trap, whilst the upstream gate is closed. However, is also possible that mitten crabs, depending on their size, are able to enter the trap through small gaps or the bars themselves (32mm spacing) of the upstream gate while the trap is fishing. It is therefore not possible to determine from which side the crabs have entered. Knowing this may provide greater insight into mitten crab behaviour during migration and also lead provide greater opportunities for mitigation options in the future.

To record the contents of the trap, an upstream barrier is lowered to stop the flow of water enabling staff to enter the trap. Water is also pumped out of the trap to reduce the water level making it easier to catch the fish for tagging and data recording. All trapped fish are recorded, and any Chinese mitten crab are removed from the trap, sexed, and then killed on site by placing them in a freezer.

Recording & Storage

Collected mitten crab specimens were stored (frozen) in containers labelled with gender, date captured, carapace width and weight (Figure 18). Specimens were then sent to NRW in Bangor for long term storage and future analysis.

Figure 18. Storage container for Chinese mitten crab marked with relevant data (sex, size, weight)

Megalopa sampling

Sampling was carried out by two members of staff, at Mostyn Docks (MD), grid reference SJ 15568 80976, and Connah's Quay (CQ), grid reference SJ 29682 69868; (Figure 19 & 20).

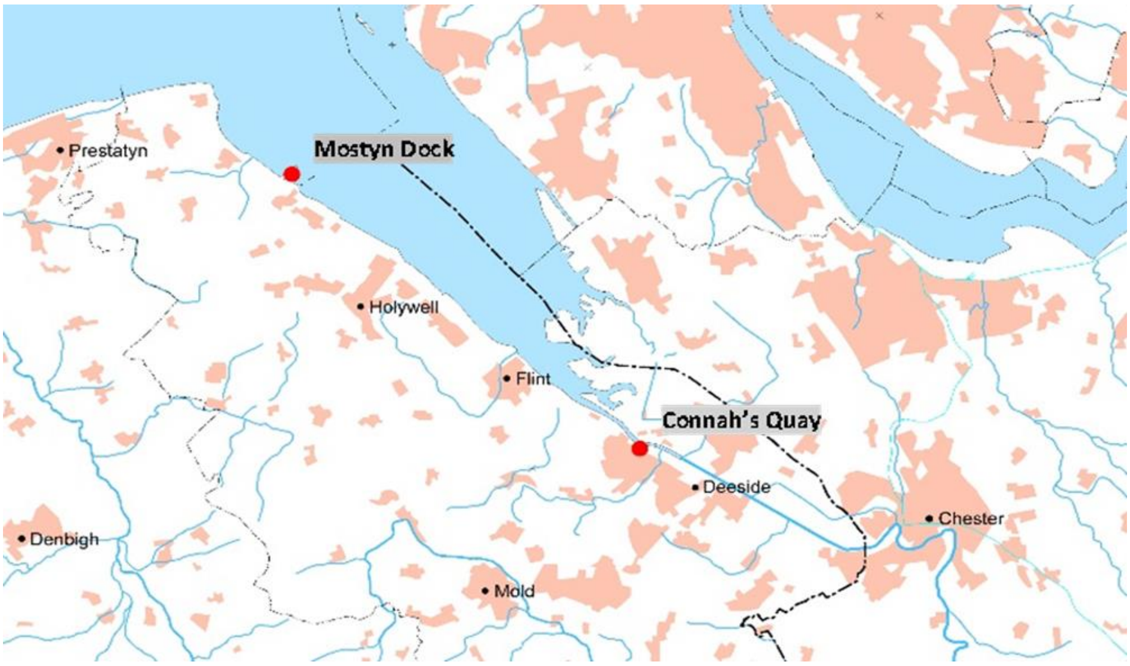

Figure 19. Megalopa collector locations (Red dots) in the Dee estuary.
Mostyn Dock (MD) is located on the western shore of the estuary between Holywell and the estuary mouth. Connah's Quay (CQ) is located immediately upstream of the estuary head, between Deeside and Flint. © CNC/NRW. All rights reserved. Contains Ordnance Survey Data. Ordnance Survey Licence number 100019741. Crown Copyright and Database Right (2015)

Successful mitten crab mating and spawning requires salinities >5psu and is optimal >10psu. Furthermore, mitten crab larval survival requires salinities from 10 to 32psu, and with an optimal range between 15 and 25psu (Anger, 1991). Consequently, abundance of larvae and megalopae may vary according to the salinity gradient of an estuary. Collector deployment sites were chosen to sample this potential variation: MD being at the mouth of the estuary, with saline conditions, and CQ being the point at which conditions start to turn to fresh water. As mitten crabs prefer saline conditions

(>20ppt) for mating and spawning, any sampling further upstream would have been unsuccessful. Sampling sites also required a suitable structure from which the collectors could be deployed to a sub-tidal depth, and that would be accessible but secure from disturbance. At MD the collector was deployed from a short floating pier, ensuring that the collector was positioned below the low tide water line. Access to the pier required permission from Mostyn Port Authority and was secured behind a locked gate. At CQ the collector was deployed from a boat moored to the quay ensuring constant submersion. The boat was accessed via a lockable ladder and therefore secure from interference.

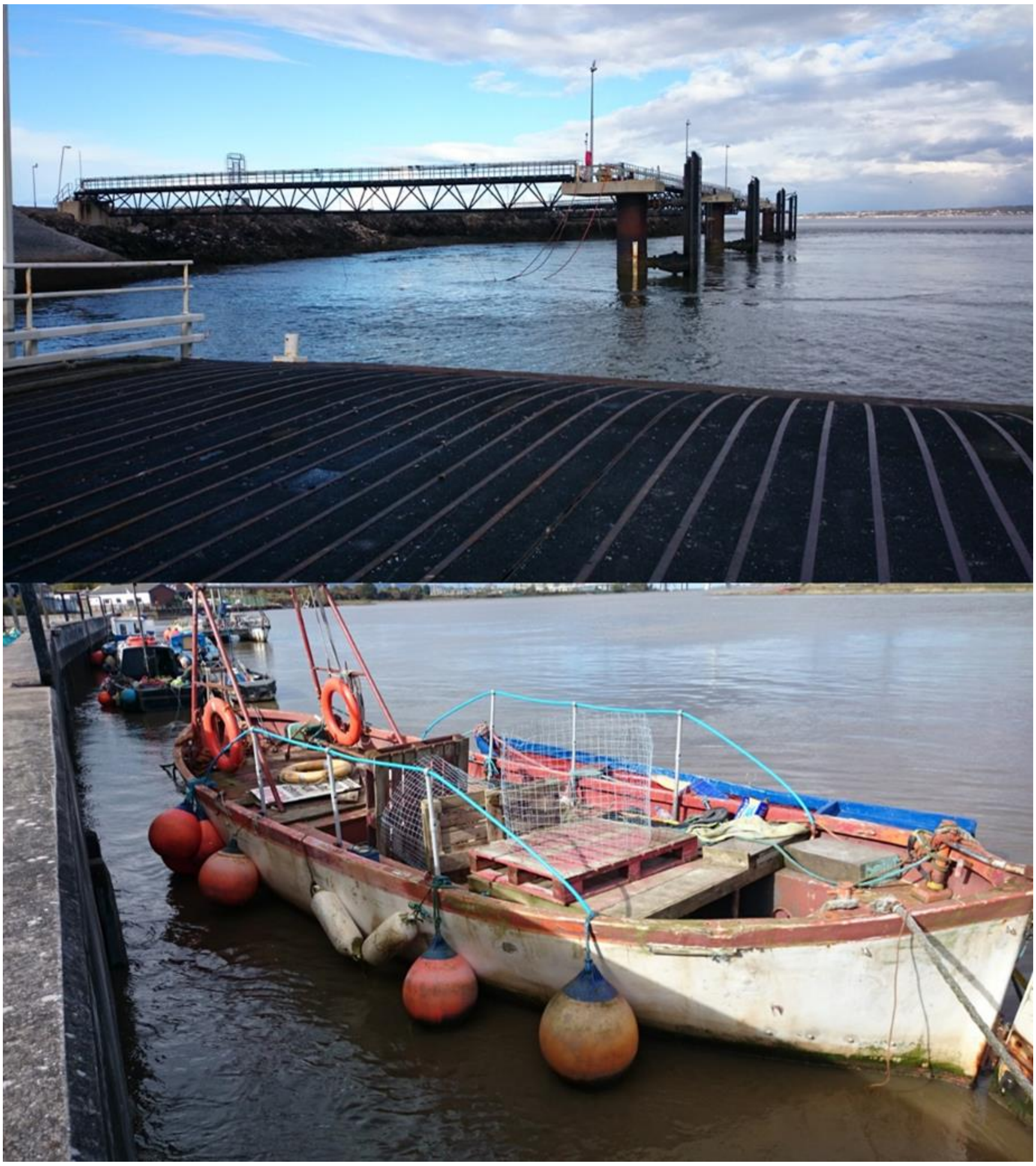

Figure 20. Megalopa collector deployment sites locations: Mostyn Dock (top) and Connah's Quay boat (bottom)

Sampling timing
Mating takes place from autumn to early spring, however the duration of embryonic development and the precise timing of hatching remain unclear. It has been suggested that, in colder waters, embryonic development is delayed so that hatching occurs when water temperatures become more favourable for larval survival (Rudnick *et al*., 2005). Successful development from hatching to metamorphosis into the juvenile crab stage requires water temperatures above 12°C (Anger, 1991).

These conditions occur in the Dee estuary between May and October (Figure 28). Larval development from hatching to the megalopa stage takes approximately 18 to 74 days (Rudnick *et al*., 2005). Therefore, megalopa sampling was undertaken from May to November at Mostyn Docks, and early June to November at Connah's Quay, to sample the period when mitten crab megalopae were expected to be present.

In previous studies of other species using similar megalopa collectors, peak numbers of megalopae were detected immediately after spring high tides. It was thought that the stronger currents into an estuary, associated with these tides, washed in more planktonic megalopae from the estuary mouth and adjacent sea (Paula *et al*., 2001). In light of these findings the megalopa collectors set in the Dee estuary were collected approximately every two weeks, at spring high tides. This was expected to allow optimal settlement of megalopae onto the collector material. Furthermore, both collector deployment sites were most easily accessed at high tide.

Megalopa collectors
Collectors were constructed of closed cylinders of plastic mesh containing a collector material. Advice from Bangor University was that the use of air conditioning material inside the collectors would be appropriate for megalopa settlement, however, this proved difficult to source. Initially, unfolded plastic scourers were trialled against the air conditioning material. These proved to be as effective, and so these were used as a substitute for the remainder of the sampling (Figure 21).

Figure 21. Plastic scourers used as collector material for settlement of Chinese mitten crab megalopae (bottom right – original form; top left - unfolded form)

At each site the collectors were deployed in threes. The scourers were supplied in multiple colours so, to control for any megalopa preference for settlement on different material colour, each of the three deployed collectors contained a different colour material. This colour was recorded at deployment and on the sample containers following collection.

Sample collection and storage
Separate containers were used for the samples from each of the three collectors deployed at each site. Sample containers were labelled with the collector site (i.e. MD or CQ), collection date, collector material colour, and staff member's initials. Samples were fixed in 1 litre of 5% formalin solution, buffered with 5g of borax, for long term storage before analysis. For laboratory protocols see Appendix 10.3.

Laboratory analysis
To analyse collected megalopae, the collector material was rinsed with water to dislodge settled megalopae. This water, and the formalin solution from the storage container were both filtered through a 250µm filter to extract suspended megalopae and juvenile crabs. Species and life history stage (megalopa or juvenile crab) were determined under a microscope using identification keys. The total number of each species and life history stage were recorded for each sample. This work was undertaken by Kate Cooper, a Bangor University M-degree student, and written up as a separate piece of work. See Cooper, 2015 for full details.

Data Loggers
The data loggers used were Star-oddi DST CTD miniature salinity, temperature & depth loggers (Figure 22). Conductivity was as recorded as a proxy for salinity. All loggers were calibrated to measure expected environmental conditions throughout the sampled portion of the river: a range of -1°C to +40°C +/-0.1°C for temperature, 3 to 37 mS/cm +/-1.5 mS/cm for conductivity, and 1m-100m +/-0.4% of selected range for depth.

Figure 22. Star-oddi DST CTD data logger.
Source: www.star-oddi.com

Ideally, environmental data collected by the loggers should be as continuous as possible. To minimise data gaps during data retrieval and staff time requirement, a portable tablet (running SeaStar data logger reading software) and battery powered data logger communication box were purchased to allow data retrieval in the field.

Each data logger was securely contained within protective plastic housing using cable ties and then securely attached to a chain, at the length required to hold the logger just above river bed (Figure 23). The positioning of the data loggers immediately above the river bed was intended to record environmental conditions at depths inhabited by Chinese mitten crab.

Five sites were identified to deploy the data loggers (Figure 24). Locations were chosen to monitor conditions throughout the lower portion of the Dee and its estuary, where *E. sinensis* may migrate and reproduce. Sites were also selected to be inaccessible by the public, to reduce the risk of disturbance from the public or other river users.

Figure 23. Protective plastic housing and attachment to chain.

Cable tie visible at the top left of the image was used to mark the logger attachment position to ensure consistency between deployments.

Chester, boat mooring (Grid reference SJ 40 65): The logger was attached to a pontoon, in the middle of the river channel, approximately 100m upriver of Chester weir. As the site is upstream of the weir, it is only tidally influenced around high spring tides. Therefore, the data logger was intended to measure conditions in fresh water mitten crab habitat.

Chester, United Utilities (Grid reference SJ 40 65): This data logger was positioned at a secure water abstraction point on the river bank at the northern end of Chester weir. This data logger measured river conditions immediately above Chester weir. It was originally intended that a data logger would be placed immediately below Chester weir to assess the effect of the weir on river salinity, however, no suitably secure location was identified.

Crane Wharf (SJ32 68): This data logger was attached to supports for a walkway along the river bank approximately 200m upriver of Crane Wharf NRW site and was only accessible by boat. This logger was placed to measure conditions in habitat suitable for juvenile mitten crabs.

Sandycroft (SJ39 66): The Sandycroft logger was attached to a derelict wharf on the bank of the canalised section of river, downriver of Chester and approximately 6km upstream of the estuary head. The data logger measured conditions at the river bed immediately below the low tide line and consequently was only submerged in very shallow water at low tides. This data logger was intended to measure conditions in brackish mitten crab habitat.

Mostyn (SJ15 81): The data logger was located to measure conditions at Mostyn Dock, on the western edge of the estuary, approximately 6km from the estuary mouth. The logger was positioned at a sub-tidal depth from a short pier. The data logger was placed to measure estuary conditions, particularly in relation to the environmental requirements of mitten crab larvae.

Each logger was marked with a serial number which corresponded to the file names of its data output. The same logger was always used at each site therefore these serial numbers were recorded to identify data from individual sites.

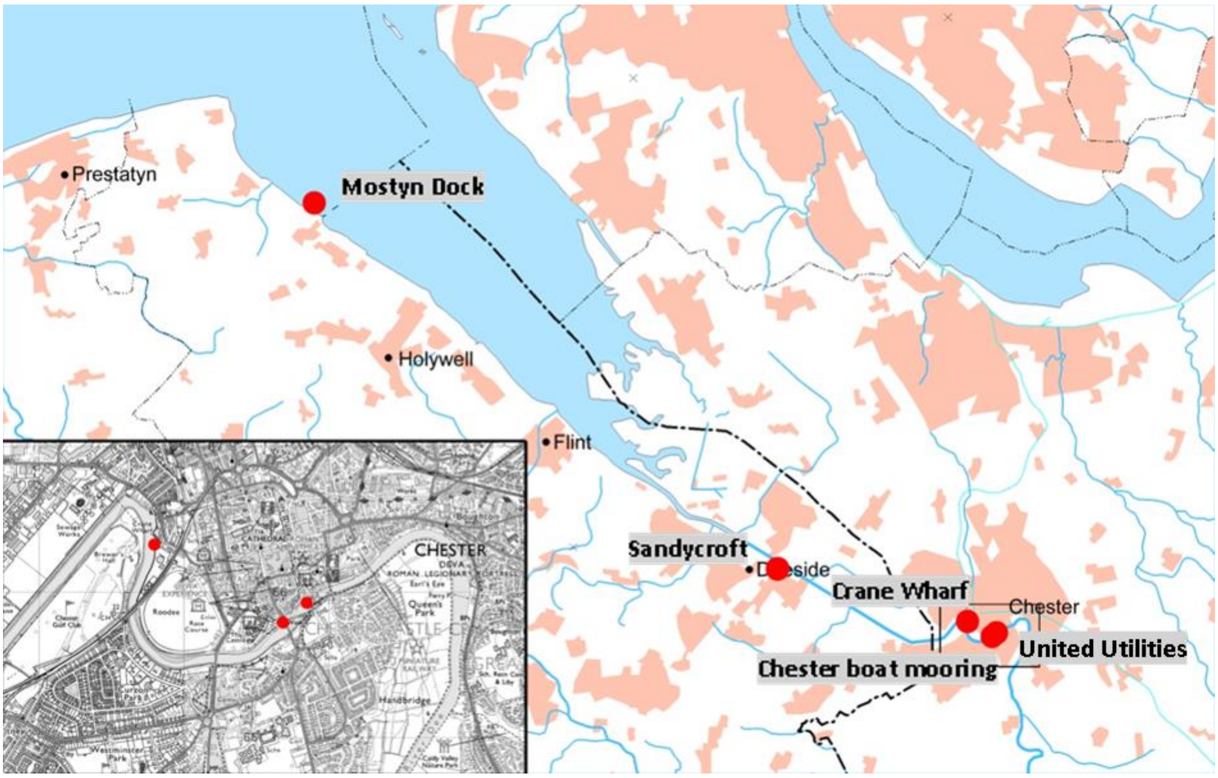

Figure 24. Data logger deployment locations (red dots).
The Mostyn Dock data logger is positioned on the western shore of the estuary between Holywell and the estuary head. The Sandycroft, data logger is positioned in the canalised Dee near Deeside. Crane Wharf, United Utilities and Chester boat mooring sites are shown in the smaller scale map (location indicated by box). © CNC/NRW. All rights reserved. Contains Ordnance Survey Data. Ordnance Survey Licence number 100019741. Crown Copyright and Database Right (2015)

Data loggers were set to record continuously and indefinitely from May 2014, taking readings of all variables every 15 minutes. Data was retrieved approximately every two months and the time the logger was lifted for data retrieval and the time it was reset was recorded for each location (Protocol included in Appendix 8.2).

6.3. Results and discussion

Juvenile mitten crab shoreline searches

Including the preliminary surveys, ten shoreline surveys were conducted between the 7th July and 31st October 2014 at four sites downriver of Chester Weir (Table 9). A total of eleven mitten crabs were recorded across all shoreline surveys. No mitten crabs were detected at Saltney and Sandycroft; however 77 shore crabs (*C. maenas*) were recorded at Sandycroft. Ten *E. sinensis* were recorded at Crane Wharf, and one *C. maenas* was also recorded at this site. One individual of *E. sinensis* was recorded at Handbridge. Five of the recorded *E. sinensis* were alive when found, the rest were dead. A number of additional moulted carapaces were also found at the Crane Wharf site. This finding reflects those of Schmidt *et al*., (2009) who conducted similar surveys on tributaries of the Hudson River, USA, and detected significantly greater numbers of moulted carapaces and dead crabs than live individuals.

Table 9. Shoreline survey dates, survey effort and outcomes by location.
** - Survey area estimated from aerial imagery (NR. – Not Recorded).*

Survey Site	Date	Survey Duration (min)	Area (m^2)	Refugia Checked	E. sinensis	Other recorded species	Surveyors and notes
Crane Wharf	03/07/2014	60	200*	NR.	7	Carcinus maenas: 1	Preliminary survey
	03/10/2014	50	200*	71	1		AF, JY.
	31/10/2014	41	200*	164	2	Anguilla anguila: 1	AF, JY, KC.
Saltney	03/07/2014	NR.	NR.	NR.	0	Carcinus maenas: 1	Preliminary survey
Handbridge	07/08/2014	NR.	NR.	NR.	1		Preliminary survey
	03/10/2014	18	72	35	0		AF, JY.
	31/10/2014	12	NR.	101	0		AF, JY, KC.
Sandycroft	19/09/2014	20	150*	27	0	Carcinus maenas: 10	AF, JY.
	17/10/2014	23	250*	50	0	Carcinus maenas: 28	AF, JY.
	31/10/2014	50	520	125	0	Carcinus maenas: 39	AF, JY, KC.

Mitten Crab Size and Age

Recorded carapace widths of captured crabs during all surveys ranged between 8 and 25mm. Data on growth rate from Rudnick *et al*. (2005) seems to indicates that the Dee juvenile crabs are likely to be between 75 days to 1 old year since metamorphosis from megalopae. However, these ages are likely to be an underestimate of actual age as they were calculated from crabs grown in 17°C laboratory conditions. For most of the year water temperatures in the River Dee and estuary are below 17°C so growth rates are probably slower. These estimated ages do indicate, however, that juvenile crabs recorded in the shoreline surveys could potentially be from more than one year class.

Intraspecific competition with C. maenas

It has been suggested that there is direct intraspecific competition between *E. sinensis* and *C. maenas* for both food and refugia. E. sinensis have also been shown to outcompete *C. maenas* for refugia in laboratory experiments (Gilbey *et al*. 2008). The two species were found to coexist at Crane Wharf, however juvenile *E. sinensis* must pass the Sandycroft and Saltney sites on their upstream migration to freshwater habitats, so it could be expected that intraspecific competition for refugia could also occur at these sites. The data from the current shoreline surveys is not sufficient to draw any conclusions regarding interactions between *E. sinensis* and *C. maenas*, however, more rigorous, systematic shoreline surveys conducted at these sites over a number of years may be more informative.

Difference in number recorded in summer and autumn surveys
Up to 70% of the *E. sinensis* recorded at Crane Wharf were found during the July preliminary survey. Juvenile mitten crabs are at highest densities and most actively moving up river during June and July (Panning, 1938). Furthermore, Gilbey, *et al*. (2008) attributed lower numbers of *E. sinensis* found during winter intertidal surveys to the crabs seeking refuge in deeper water during colder months. The difference in numbers detected during the preliminary shoreline surveys in July and those conducted later in the year in the current study seems to reinforce these findings, although due to the limited number of surveys undertaken and numbers recorded this data is inconclusive.

Quantifying sampling effort
In order to allow meaningful comparisons between surveys conducted at different sites, between different times of year and between years, survey effort must be accurately quantified. Survey duration, area surveyed and number of refugia checked were all recorded as measures of sampling effort. Survey duration varied due to a number of factors and is unlikely to have been representative of sampling effort. The terrain varied considerably between sites. Sandycroft and Saltney were flat and sandy so surveyors could move across the site with ease. Whereas the steep, muddy banks at Crane Wharf required greater care to be taken when moving across the site requiring more time to survey an equivalent area.

Area surveyed would allow comparison between surveys of the same site however would not be ideal for comparisons between sites as density of refugia may vary between (and within) sites, and would require a GPS or tape measure to accurately record. Number of refugia checked provides an easily measurable way of quantifying sampling effort in a consistent manner between sites and surveys. The value for each survey is unaffected by ease of movement across a site, variation in densities of refugia and time taken to record data. It also accounts for differing numbers of surveyors and is simple to record, requiring no extra equipment. However, it does not take into account variations in the size of refugia which could shelter differing numbers of crabs, although rock size did not appear to differ greatly between each site.

Habitat quality of sampling sites
In San Francisco Bay, juvenile *E. sinensis* are associated with a wide range of habitats but were most common in low salinity (<6psu) areas with steep, vegetated clay or silt banks. Juvenile and adult mitten crabs are often noted for their burrowing behaviour; however burrowing was only recorded on banks steeper than 35° made of a suitable substrate. In the presence of other suitable refuges in the habitat, or absence of suitable banks for burrowing, mitten crabs are flexible in their refuge preference (Rudnick, 2005). Juvenile crabs in particular have been recorded using vegetation and rocks on the substrate surface in the intertidal zone (Gilbey, *et al*., 2008, & Schmidt, 2009). Gilbey *et al*. (2008) found that crabs occupy rocks as refugia throughout the entire intertidal zone with no significant preference for upper or lower shore levels, potentially occupying any refuge within the intertidal zone. This implies that differing densities of refugia at different shore levels between sites should not

affect the likelihood of recording mitten crabs, nor should the area of the intertidal zone. The Sandycroft site had a relatively flat intertidal zone with sandy substrate and rocks on the surface. The site has ample suitable refugia which were found to be used by *C. maenas* and would be suitable for *E. sinensis*.
Salinity fluctuated between 0 and 27psu dependent on tides (measured using the nearby data logger), with an average salinity of 5psu. Average salinity is within the preferred range reported by Rudnick (2005); however salinity of the incoming tide far exceeds the average. Juvenile mitten crabs settle out of the water column from megalopae throughout the estuary and migrate upstream to brackish and fresh water. Due to the high peak salinity juveniles should migrate upstream through this location but may not be present after the summer migration period. Surveys at Sandycroft were conducted from mid-September through October, which may explain the absence of recorded *E. sinensis.*

The Crane Wharf site had a steep, muddy bank with overhanging vegetation and rocks concentrated towards the low water line in a 1m to 2m band. Salinity, measured by the data logger immediately upriver from the site, was predominantly low (≈0psu) but peaked to 10psu during spring tides. This habitat more closely resembles the optimal conditions outlined by Rudnick (2003). The majority of *E. sinensis* found were recorded at this site.

At the Handbridge site, habitat varied from soft mud immediately below the NRW fish trap at Chester Weir, with sparse rocks on the surface and vegetation close to the high water mark (submerged during spring high tides), to gravelly with larger rocks on the surface, close to and under the bridge. Salinity, measured by a data logger above the weir, was 0psu (except at particularly high spring tides when the data logger recorded a maximum of 3psu). However, salinities below the weir may be greater than measured by the data logger as the weir prevents most upriver flow of brackish water. The muddy substrate and the presence of rock refugia and vegetation appeared to be favourable juvenile mitten crab habitat, however only one mitten crab was recorded at this site in July and none in subsequent surveys. It is possible that the vegetation to the rear of the site may have been utilised by juvenile mitten crabs as a preference due to limited rock refugia, however this was not investigated as part of this study.

Effects of repeated sampling of the same sites
The survey method required some disturbance of shoreline habitat and therefore repeated surveys of the same site may degrade the habitat over time and reduce the likelihood of detecting *E. sinensis* in future surveys. To reduce the effect of disturbance, all refugia were replaced in their original position after being checked. *E. sinensis* were recorded in consecutive surveys of the same site (Crane Wharf) indicating that they are able to re-colonise sites between surveys, despite any disturbance caused. This is supported by Gilbey *et al*., (2008) who reported high levels of movement of juveniles in the Thames intertidal zone and influx of new crabs to surveyed sites with each tide. However, as *E. sinensis* are less abundant on the Dee, influx of new juveniles to survey sites may be limited.

Recorded numbers of *E. sinensis* were greater in the preliminary surveys, however higher numbers of juveniles were also recorded during the summer months by other studies in the literature, which describe seasonal behavioural differences which account for the change in abundance (Gilbey *et al*., 2008).

Summary
The shoreline Chinese mitten crab surveys conducted during 2014 successfully detected low numbers of the species at two locations. The method is suitable for determining presence/absence at surveyed sites and, if applied in a rigorous and systematic manner, could be used to measure: relative abundance between sites; changes in seasonal abundance at, and between sites; and measure longer term population trends. Similar methods have also been used in conjunction with mark and release methods to measure migration rates. Quantification of survey effort allows the data collected during the 2014 surveys to act as a baseline against which to compare future surveys. The methodology has the advantages of being simple to conduct and requiring little equipment. However these surveys are only possible in tidal areas of the river and so, on the Dee, are limited to areas below Chester Weir.

Surveyed sites were easily accessible and represented a range of habitats in the Dee upper estuary. Volunteers assisted with some of the surveys. Due to the simple methodology and accessibility of sites this survey method may be a suitable activity for larger public engagement events

Although successful at detecting juvenile Chinese mitten crab, these intertidal shoreline surveys only detected low numbers of individuals and are only possible in tidal areas of the river. These factors may result in under-recording of juvenile presence and abundance. Investigation of alternative sampling methods may yield better data. For instance, Rudnick *et al*. (2005) deployed artificial shelter traps consisting of multiple stacked tubes (6 inches in diameter). These were used to sample juvenile sized crabs, over 5mm carapace width. These traps could be deployed at sites sampled by this project but also from the river banks in the non-tidal Dee. Such a monitoring technique was suggested by NRW staff early in the project but lack of resources and other priorities meant that this could not be delivered as part of the current project.

Fyke Netting
Modified fyke nets without wings were deployed on six dates between the 12th September and the 18th October 2013 at Chester Weir, Eccleston and Aldford (Table 7). Another net was deployed continuously at Holt between the 17th September 2013 and November 2013 (checked daily). The nets were set for approximately 24 hours soak time per deployment (average: 22 hours). No mitten crabs were trapped during any deployment.

During 2014 a modified fyke net was successfully deployed at Crane Wharf on five occasions between the 18th August and the 24th October. Nets deployed on the 18th August, the 20th and 26th September remained in their correct position until checked and retrieved (Table 8). It should be noted that a large tree limb became entangled in the net retaining rope on 20th September. Despite this the net remained in its intended position.

Further attempts were made to set the net on the 17th, 21st and 24th October, however these were unsuccessful due to unfavourable river conditions: increased rainfall during October caused increased river flow; consequently it became unsafe to deploy the net from the boat, as it was not possible to maintain control of the boat throughout deployment. Mean soak time of successful deployments was 23 hours 33 minutes. No mitten crabs were trapped during any deployment. One eel (*Anguilla anguila*) was found in the net on the 19th August during a preliminary check before retrieving the net, which subsequently escaped.

Although fyke nets have been successfully used in similar studies on the Thames and Guadalquivir (Garcia-de-Lomas *et al*., 2010), no crabs were trapped by any deployed net in either 2013 or 2014, calling into question the suitability of using fyke nets for trapping Chinese mitten crabs on the River Dee. However, the apparent lack of success could be attributed to a number of factors. For instance: differences in the methodologies used compared to previous studies; inappropriate deployment locations; and insufficient sampling effort to detect Chinese mitten crabs at their current numbers.

The 2013 methodology differed from previous successful use of fyke nets in that the 2013 nets were deployed without wings (a stipulation of the license) and were situated close to the river banks from which they were set. This effectively only sampled a very small section of the river channel. The 2014 methodology more closely resembled the successful protocols used by Clark (2008), on the Thames. Nets, with wings attached, were deployed close to the centre of the river channel, sampling a much greater width of the river channel than in 2013. The river is fairly narrow at this sampling point (approximately 40m) so a successfully deployed fyke net, with wings, spanned over a quarter of the width of the river. Additionally, the deployment location (Crane Wharf) was further downstream than any of the 2013 locations, downstream of Chester weir where migrating adults mitten crabs have been successfully recorded. Due to these factors and the lack of any other tributaries leading down into the estuary from this point, it was postulated that all migrating adults would have to pass this location in order to enter the estuary.

During the course of the trial monitoring activities a number of observations were made regarding the potential effectiveness of the methodology used which have been outlined below:

- In order to deploy the net with the intended “V” funnel configuration for the wings (Lake, 2013), it was necessary to allow a large amount of slack in the retaining ropes due to the size of the net. This was fine during the ebbing tide, however, once the tide turned the force and speed of the flooding tide, coupled with the slack in the ropes caused the net to lift where the leader was tethered to the river bank.
- The wings, although weighted, may not have remained fully in contact with the river bed throughout the tidal cycle
- The conical net anchor was demonstrably not sufficient to consistently maintain the net’s deployed position in high flow rates and against incoming tides causing inversion, twisting and washing of the entire net up river.
- The net could only be deployed safely from a boat or kayak at low slack tide, limiting when it was possible to set and retrieve the net, particularly later in the year when there were limited daylight hours and increased river flows due to high rainfall.

- It is also likely that the shape of the entrance of the net (circular) was not ideal for trapping benthic species. Future studies should try utilisation of fyke nets with 'D' shaped openings which would increase contact between the opening of the net and the river bed.

Setting fyke nets in stronger currents can be problematic (Lake, 2013). Alternative methods of anchoring the nets to the river bed may help to alleviate these issues. One such method could involve driving rigid stakes directly into the river bed to anchor the leaders and the cone end. These stakes are less susceptible to movement in stronger currents, and direct attachment of the leaders helps to prevent twisting of the net. However, this method is only possible in relatively shallow water or fully intertidal areas and was not possible at the Crane Wharf site.

Potential effects on protected species through by-catch

One concern about the use of fyke nets to monitor *E. sinensis* on the Dee was the potential to trap other, protected, species as by-catch. During the 2013 trapping period five flounder (*Platichthys flesus*) were caught as bycatch, of which one was still alive when the net was retrieved. Flounder are not a protected species on the Dee and therefore this by-catch was deemed acceptable. In 2014 only one live eel (*Anguilla anguila*) was found on initial inspection of the net but had escaped by the time the net was retrieved minutes later. This suggests that the 40mm mesh on the cone end of the net was effective mitigation to reduce impact on this species.

Summary

Despite the failure of modified fyke nets on the Dee to capture Chinese mitten crab, their success in other studies and having evaluated potential reasons for their not having detecting *E. sinensis*, they may still be a viable tool for monitoring the species in the Dee catchment in the future, perhaps if the population increases. Measures taken to reduce the risk of harm to protected species appear to have been adequate and suggest that future use of fyke nets poses an acceptable level of risk to these species. Similar methodologies to the Clark (2008) and Garcia-de-Lomas *et al*. (2010) could be applied but at less tidal locations or with an alternative anchoring method. Alternative trapping methods (such as crab pots) could also be trialled.

Trammel netting

A local well known fisherman, provided records of mitten crab for fisherman generally under the raising awareness section of the project. He was also paid to take a boat out into the Dee in order to undertake some trammel netting work in 2013. Trammel netting for Chinese mitten crab was undertaken on 4 separate occasions that coincided with the detection and movement of adult crabs obtained from the NRW fish trap. However, out of the four trammel netting sessions only 3 adult mitten crabs were captured (all during the same session).

Chester Fish Trap

Mitten crabs were first recorded at the trap in 2007, with four individuals trapped between August and November. Total numbers recorded at the fish trap fluctuated between 21 and 5 individuals between 2008 and 2012. In 2013, the number of recorded mitten crabs increased to 82 individuals and remained at a comparable 76 individuals in 2014 (Figure 26). In the years 2007 to 2014, 94% of recorded crabs were trapped during the months of August, September and October with peak numbers recorded every year in September. The timing of these records are very similar to the timing of adult downstream migrations recorded for other populations (Rudnick *et al*., 2005), and may indicate that the vast majority of crabs recorded at the fish trap are passing the trap whilst migrating from further upstream.

Morrit *et al*. (2013) found the sex ratio of mitten crabs collected from a power station water abstraction point on the Thames to be skewed toward males. At the NRW fish trap, sexes of mitten crabs were not recorded before 2013. In the 2013 and 2014 data interestingly there is almost no difference between the numbers of males and females with averages across both years being 39:35 and 39:36 respectively (Figure 25, graph shows 2013 results only).

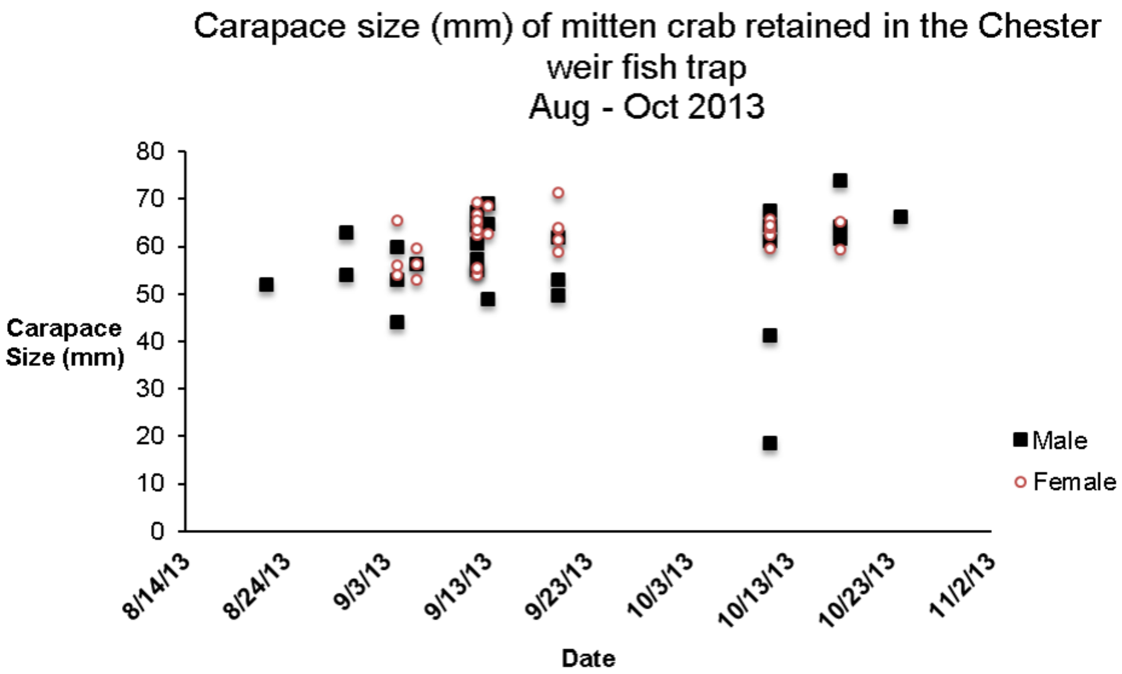

Figure 25. Carapace width (mm) of male and female Chinese mitten crabs recorded in the Chester weir fish trap during 2013.

The graph shows live crabs only. Total numbers of live and dead crabs were: males = 42 and females = 40.

Carapace width and weight were recorded for individuals trapped during 2013 and 2014. Carapace width ranged between 18.7mm and 76.6mm (mean carapace width: 61.2mm) during the two years. There was little difference in mean carapace width between the two years (2013: 60.5mm; 2014: 61.9mm). Weight ranged between 8g and 215g (mean weight: 125g) across both years. Mean recorded weight dropped from 131g in 2013 to 118g in 2014. However, differences in the weighing protocol may account for this difference. The weights of crabs trapped in 2013 were recorded at, or soon after, capture, before freezing for storage. Weights measured in 2014 were recorded from specimens which had been stored frozen and thawed prior to weighing. During the thawing process some liquid appeared to be lost from the specimens. Consistent with studies of other mitten crab populations, there was no apparent size or weight difference between male and female crabs. Based on growth rates reported by Rudnick *et al*. (2005), and Herborg *et al*. (2003) (12mm.year^{-1}), the range of carapace size of crabs recorded at the fish trap indicate that these crabs may be aged from <1 year up to a maximum age of 5 years and therefore appears to trap juveniles in addition to reproductive adults. In a parallel study, conducted at Bangor University, of physiology of the specimens trapped at the fish trap during 2013, Cooper (2013) found no reproductively mature specimens measuring below 48mm. This further supports that both adults and juveniles are sampled from the fish trap, possible indicating that the trap is detecting juveniles moving upstream as well as adults migrating down.

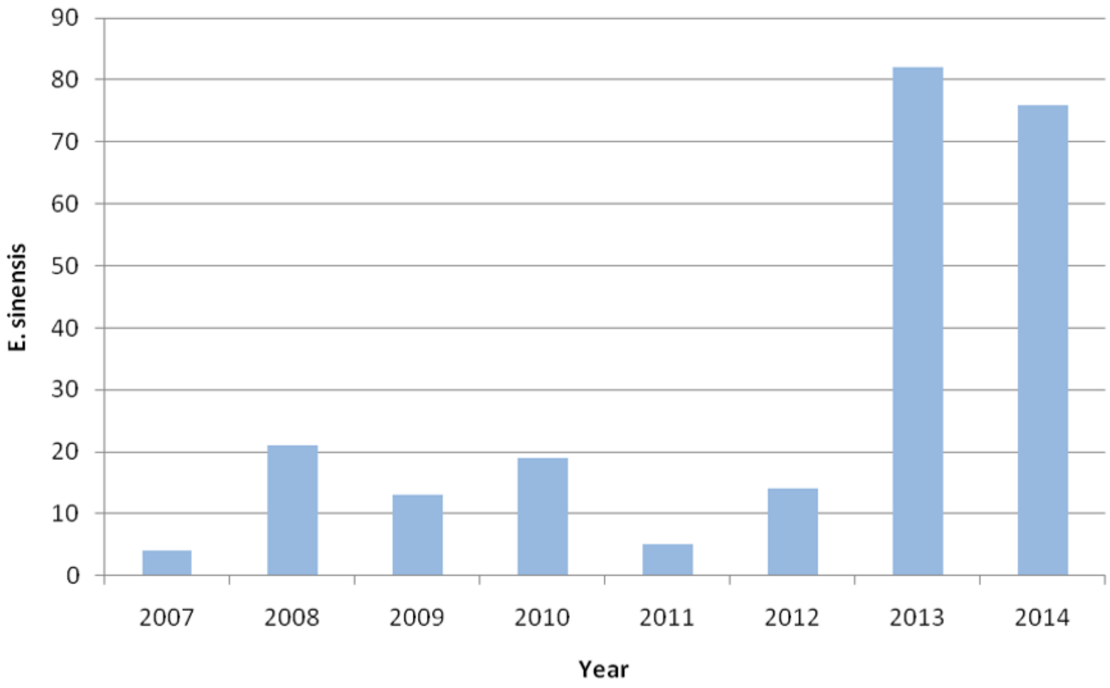

Figure 26. Total abundance of *E. sinensis* trapped at the NRW weir Chester fish trap for each year between 2007 and 2014.

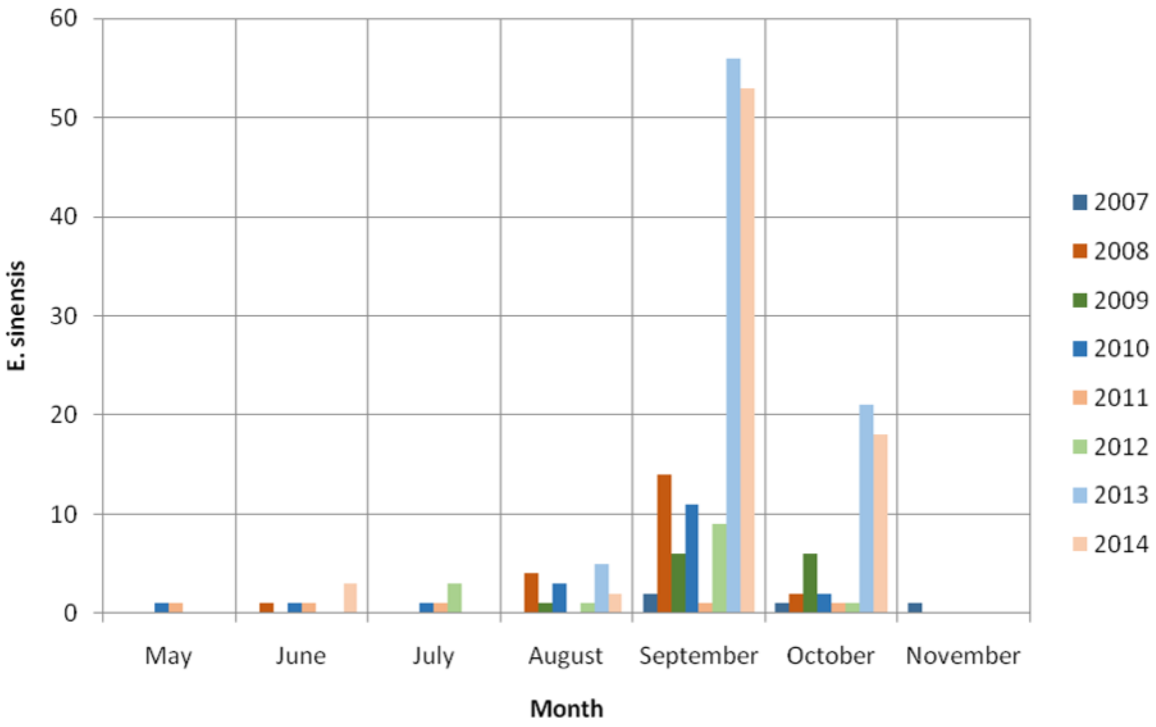

Figure 27. *E. sinensis* trapped at NRW fish trap, Chester Weir by month for years 2007 to 2014.

Sampling effort

Trapping is carried out throughout the year but not continuously. The trap was fished for 4421 hours in 2013 and 4336 hours in 2014. There was some variation in fishing time between months, however monthly average fishing time differed during the August to October migration in 2013 and 2014; 368 and 358 hours respectively. This difference of 2.6% could have contributed to the difference in recorded numbers of mitten crabs between the two years. However, it is also worth remembering that any crabs in the trap are able to exit through the downstream entrance (where the fish enter) so crab behaviour may also contribute to variation in numbers.

Exact data was not provided for sampling effort at the fish trap prior to 2013, however, due to the use of the fish trap for long term Salmonid monitoring, sampling effort between years is assumed to be roughly equivalent. The slight variation in sampling effort may account for subtle changes in recorded numbers between years but is unlikely to have affected recording of gross relative abundance. The increase in recorded numbers of mitten crabs at the fish trap between 2012 and 2013 coincides with the timing of this project, when the fish trap staff were specifically asked to collect the species. Increased vigilance for the species may have contributed to the increase in records from 2013 however it is unlikely that this factor alone accounts for significant increase in numbers.

Properties of the fish trap favourable to Chinese mitten crab

The NRW fish trap at Chester weir has been more effective at detecting Chinese mitten crabs than any other methodology trialled. Further investigation of the properties of the fish trap, which make it favourable to *E sinensis*, could help refine other trapping methodologies to make them more effective at trapping the species.

As the majority of recorded *E. sinensis* appear to be migrating adults (Figure 27), these crabs would be expected to enter the trap from the upstream side of the fish trap as they are migrating down river. This would be an unusual route for the crabs to take as the upstream opening is at the top of a vertical concrete face above an overhanging lip which the crabs are unlikely to climb. This opening is also positioned perpendicular to the river flow so is unlikely to become silted to the point where mitten crabs could enter (R. Cove, NRW, *pers. comm*., 2015). However, mitten crabs are good climbers and even a direct route over the top of the weir requires climbing up a semi-vertical face. It is also possible the increased flows around the area of the fish house due to the funnelling effect created by the trap acts as a channel for the crabs to attain easier access over the concrete face/lip.

The outflow from the trap connects to a fish ladder which circumvents the weir. It is possible that mitten crabs are moving against the flow into the trap, entering from the downriver entrance. Adult and juvenile mitten crabs are known for burrowing into muddy banks or sheltering under rocks which are both dark environments, although it is unclear whether this behaviour continues during the adult downstream migration.

Mitten crabs found in the fish trap appeared to favour the darker side of the trap with the majority of individuals found between the darker wall and metal gate opened against it (R. Cove, NRW, *pers. comm*., 2014),suggesting that the low light in the fish trap may be a contributing factor to its success at trapping mitten crab. However, it is also feasible that movement and disturbance caused by staff when processing the fish caused the crabs to hide in the darker areas of the cage.

Differences in juvenile and adult numbers

Demographically, it would be expected that juvenile mitten crabs should outnumber mature adults in terms of the numbers captured in the fish trap. However, the majority of crabs recorded at the fish trap were mature adults with relatively small numbers of juveniles detected. This implies that the fish trap is more successful at trapping adults than juveniles. However, fish trap staff have observed adult mitten crabs passing between the bars so it highly likely that any most of the juveniles are able to pass straight through. The bars on the gates of the fish trap are spaced with an opening of 32mm which, if adults are entering through the open downstream gate, may cause adults to become trapped whilst smaller juvenile crabs are able to pass through the trap.

Further investigations related to the possible mechanisms by which both adult and juvenile mitten crabs become trapped or perhaps simply take refuge in the fish trap are needed in order to establish how and why mitten crabs are entering the fish trap. These insight may provide a potential mechanism for future management measures in the future.

Summary

Records from the NRW fish trap, Chester, are the most consistent and comprehensive measure of Chinese mitten crab numbers on the River Dee. Records from the trap provide a measure of relative population size between years. Size, weight, and timing of recorded crabs suggest that the trap predominantly traps downstream-migrating adults during an equivalent period of peak migration recorded in other studies. Small numbers of juveniles are also recorded at the trap throughout the spring to autumn. The presence of both juvenile and adult age classes is supported by physiological analysis of specimens, collected from the fish trap in 2013, which found that only individuals measuring more than 48mm were reproductively mature (Cooper, 2013).

The reasons behind the differing numbers of juvenile and adult crabs recorded at the fish trap are currently unclear, however, it is likely that juvenile crabs are able to pass straight through the trap, resulting in lower numbers being recorded. Or possibly that juveniles are taking an alternative route over the weir. Sampling effort is quantifiable and past differences may account for small amounts of variation in recorded numbers of mitten crabs between years. As the fish trap is so effective in comparison to other trialled trapping methods, it may be able to inform refinement of other sampling methodologies. The dark environment within the fish trap emulates conditions in natural mitten crab refugia (e.g. in burrow or under rocks) and may therefore prove useful when designing future monitoring or management techniques to increase effectiveness.

Megalopa Collection

Megalopa collectors were set continuously from 23rd May to 7th November 2014 at Mostyn Dock and 2nd June to 7th November 2014 at Connah's Quay. Crabs and megalopae of four native species were detected at both sites (Table 10). No mitten crab megalopae were detected at either site.

Table 10. Species detected in megalopa collectors at Mostyn Dock and Connah's Quay from May – November 2014.

	Mostyn Dock		Connah's Quay	
Species	Juv. Crab	Megalopae	Juv. Crab	Megalopae
Eriocheir sinensis	0	0	0	0
Anapagarus sp.	6	10	3	15
Atelecyclus rotundus	18	0	1	0
Carcinus maenas	3416	446	3438	792
Pisidia longicornis	184	127	3	1

Possible reasons for E. sinensis megalopae absence

Relative abundance

Due to its recent establishment, the *E. sinensis* population is likely to be small in comparison to native detected species possibly resulting in the lack of detection in the current study. In the longer established Californian population Blumenshine, *et al*. (2011), reported that *E. sinensis* zoea and megalopae were sparse in comparison to other zooplankton in San Francisco Bay. Furthermore, in the Guadalquivir estuary where *E. sinensis* has been established since 1987, in greater numbers than on the Dee (Garcia de Lomas, 2010), a study of the estuarine planktonic Decapod community found *E. sinensis* to represent only 0.004% of recorded specimens (Cuesta *et al*., 2006). These findings suggest that even in estuaries with more established mitten crab populations, numbers of megalopae occur in low densities. Consequently, the 2014 megalopa sampling effort may not have been sufficient to detect *E. sinensis* megalopae at their current abundance, if they are indeed present.

Water temperature
Survival of mitten crab larvae is highly dependent on water temperature and salinity. Development to megalopa stage requires water temperature above 12°C and salinities above 15 psu (Blumenshine *et al*., 2011, Anger 1991). Water temperature in the Dee estuary is below this threshold until the beginning of May in general (Environment Agency data logger readings). Consequently mortality in zoeal stages hatched before May could be high and only a very small proportion may develop to the megalopa stage. It may be that the high larval mortality associated with low water temperatures result in abundances of mitten crab megalopae too low to be detected by the 2014 sampling effort. However, it has been suggested that *E. sinensis* may be flexible in the timing of embryonic development and delay hatching until water temperature is more favourable for larval survival.

In the San Francisco estuary Rudnick *et al*. (2005), found that highest numbers of ovigerous females occurred between November and March, but were present in lower numbers until June. Anger (1991) found that hatching occurred approximately 30 days after mating in laboratory conditions of 17°C and 20psu but this duration increased at lower temperatures. Panning (1938), reported that larvae normally hatch between March and July in north-western Germany but hatching may end earlier in warmer springs. Estuarine water temperature in the Elbe and other northern European rivers with established mitten crab populations are comparable to those of the river Dee. These other populations are self-sustaining which would suggest that the species should also be capable of completing its life cycle in the Dee.

Collector locations
Based on the euryhaline nature of first mitten crab zoeal and megalopae stage, and preferences for more saline conditions of intermediate larval stages, Anger (1991) proposed a model of larval stage distribution in estuaries. Anger's model suggests that megalopae would be expected in greatest concentrations toward the estuary head. However, currents within the Dee estuary may drive megalopae toward the mouth of the estuary and megalopa salinity tolerances preferences allow development in saline conditions.

The collector locations at Connah's Quay, at the estuary head, and Mostyn Dock, mid estuary, should have sampled differences in megalopae concentrations along the length of the estuary. However, collector location may still have contributed to the absence of detected mitten crab megalopae. Collectors were positioned on the western shore of the estuary. There may be differences in concentration of mitten crab megalopae toward the centre of the estuary and/or between the sides. For instance, if megalopae are more abundant in deeper water then collectors would not have been optimally placed. The eastern side of the estuary has extensive salt-marshes which might provide a more favourable habitat for mating adult mitten crabs. It is feasible that spawning and larval development may occur predominately on this shore (opposite the Mostyn Dock collector locations) and that megalopae would therefore be more concentrated toward the eastern side of the estuary.

Continued use of this methodology could involve deployment of collectors at additional locations representative of all conditions in the estuary, for instance closer to the estuary mouth and between the Mostyn Dock and Connah's Quay locations, and also on the eastern shore of the estuary. The feasibility of attaching collectors to buoys in the middle of the estuary could also be examined. These were all considerations during the current study but due to limited resources only a limited number of easily accessible sites could be sampled.

Duration and timing of sampling
As discussed above, mitten crab larval development can only occur at water temperatures above 12°C. As measured by the data loggers and independent Environment Agency measurements, these conditions exist in the Dee estuary between May and October (Figure 28). The timing of the 2014 megalopa sampling (April to December) covered this window. Based on the time of year that adults have been recorded in the Dee estuary and spawning times of other populations, megalopa abundance would be expected to have peaked during the sampling period. It would seem unlikely that the absence of collected mitten crab megalopae was due to incorrect timing of the sampling.

Evaluation of sampling methods and materials
Multiple collector materials and colours were trialled to assess those most suitable for *E. sinensis* megalopae sampling. Given the absence of *E. sinensis* megalopae no conclusions can be drawn about *E. sinensis* preferences for collector material properties. However, there were procedural benefits to using scourers rather than finer AC filters. The AC material used in the early collector deployments was difficult to source and had finer and more densely packed fibres which made extraction of megalopae more time consuming. Furthermore equivalent numbers of megalopae of native species settled on the AC material as the coarser scourers when deployed simultaneously (Appendix 10.4). Scourers as a collector material would be preferable to AC material in future megalopae sampling efforts. No preference for collector colour in *E. sinensis* was reported as none were detected using this methodology, however, future uses of this methodology should continue to use multiple colours of collector to determine any preference.

Summary
Detection of *E. sinensis* larvae and megalopae in the Dee estuary would strongly indicate self-recruitment to River Dee population. The detection of other Decapoda megalopae by this methodology suggests that the sampling method is suitable for general megalopa detection in the Dee estuary. The absence of *E. sinensis* megalopae in the samples is more likely due to insufficient sampling effort to detect them at their current abundance rather than their absolute absence from the Dee estuary. Collectors at a greater number of locations and distributed more widely across the estuary and in varying habitats may be required to detect *E. sinensis*, however, in general, using the collector methodology from the shore is very time consuming and fairly inefficient.

The use of plankton nets towed behind a boat (Blumenshine, *et al*., 2011) could be a more effective way to detect the presence of Chinese mitten crab megalopae. This method would have the benefit of sampling much larger volumes of water in multiple locations and would also detect other larval stages. Boat work is costly and time consuming which is not always feasible with limited project budgets, however, the feasibility of combining mitten crab megalopa sampling with other ongoing marine survey activities is something currently being investigated by NRW.

Data Loggers
The data loggers were deployed continuously from mid-April 2014 at Mostyn Dock and Chester Boat Mooring and mid May 2014 at Sandycroft, Crane Wharf and Chester, United Utilities. Data displayed in this report was retrieved up to December 2014 or January 2015, dependant on location.

Temperature
Temperature showed consistent seasonal trends across all deployed data loggers. All loggers measured an increase in temperature from date of deployment until a monthly average of 19°C in July. After July, water temperatures fell gradually until averages of around 6°C in December and January. Peak summer temperature was similar across all loggers at ≈22°C with the exception of the Sandycroft logger which peaked to 30.5°C (Figure 28). Averages and lowest temperatures in December decreased gradually from Mostyn moving upstream to the data loggers in Chester. Average temperature at Mostyn during December was 7.7°C (Low= 5.7°C) whereas the average temperature at the Chester Boat Mooring and United Utilities data loggers was 6.4°C (Low= 3.3°C).

Salinity and tide
As would be expected for the estuary, the data loggers measured an increase in average salinities downstream from the Chester loggers (annual average of 0psu) to Mostyn Dock (annual average of 22psu). River water above Chester weir is functionally fresh. Salinity peaked at 2.5psu and 3.1psu at Chester Boat Mooring in July and September respectively, however, these peaks coincided with particularly high spring tides which overtopped the weir. The measured salinity at Crane Wharf also averaged 0psu throughout the year with the exception of September when salinity rose during every high tide between the 8th and 13th to a peak of 10psu. This peak coincided with the peak salinity reading from Chester Boat Mooring. Salinity at Sandycroft was also highly tide dependent. The annual average salinity measured at Sandycroft was 5psu, although it fluctuated from 0psu up to a maximum of 26.8psu on rising tides. These readings were similar to measurements from the loggers further up river where the highest salinities coincided with the highest tides.

Sources of error
Calibration ranges
The data loggers were calibrated to read salinity to a lowest concentration of 2psu. The data loggers positioned above Chester weir predominantly recorded salinity as 0psu. Readings taken with a more sensitive salinity probe on the 19th September during an outgoing tide found salinity to be 0.2psu at Chester United Utilities, 0.1psu at Chester Boat Mooring, 0.2psu at Crane Wharf and 0.8psu at Sandycroft. These values are below the minimum threshold measured by the survey loggers and therefore are not represented in the data. The survey data loggers were able to record temperatures between -1 and 40°C and therefore all natural variations in water temperature were recorded. Measurable pressure range of the data loggers was from 0 to 100m depth and therefore all natural variation should have been recorded.

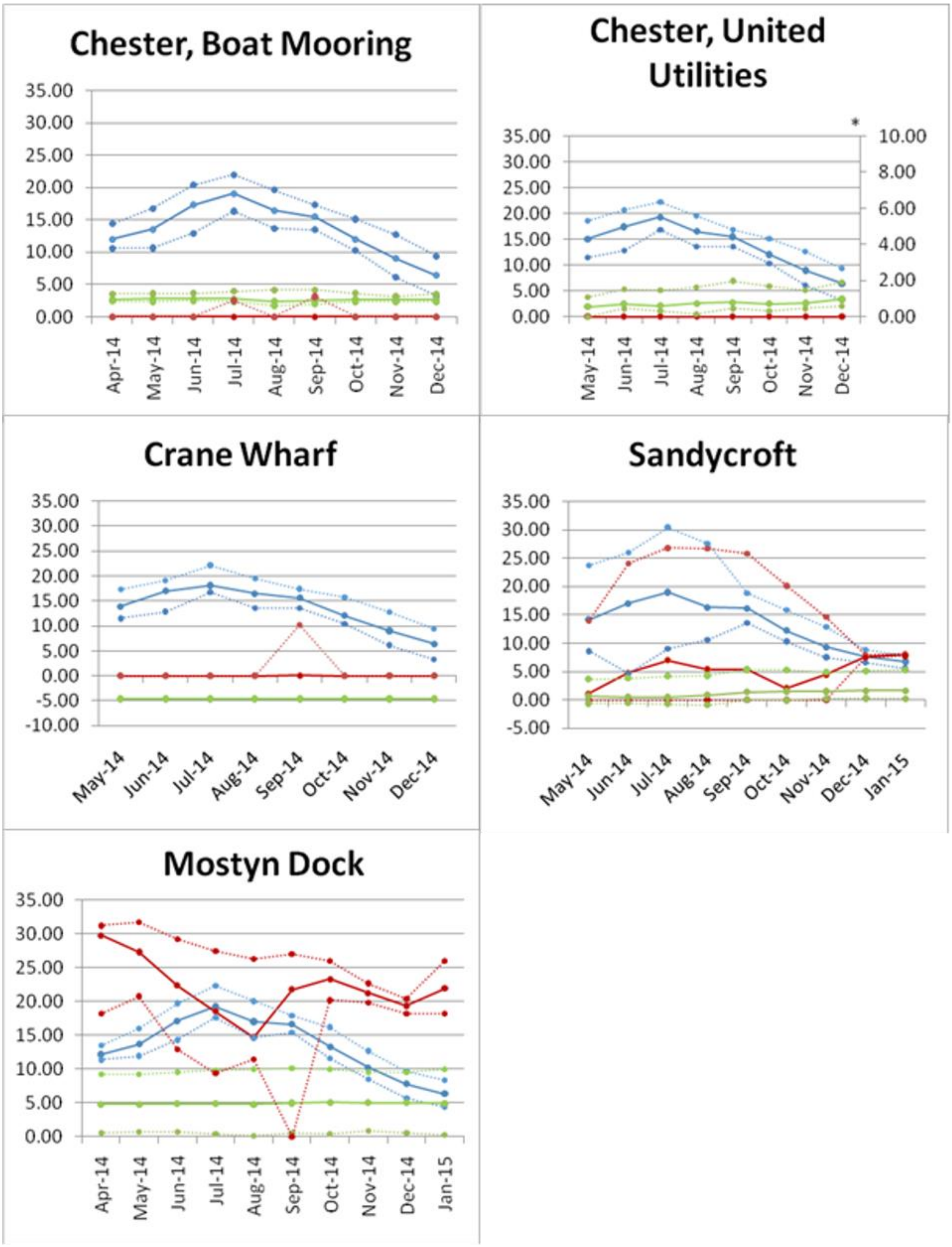

Figure 28. Monthly means, highs and lows for temperature (°C), salinity (psu) and depth (m) measured by each data logger.

Temperature is shown in blue, salinity in red, and depth in green. Solid lines represent means and dashed lines represent highs and lows for each month. Values for the first month of each graph are only representative of the time after the logger was first set, not the full month.

**The right axis for 'Chester, United Utilities' is depth (m).*

***Data logger immersed in silt from 14th November.*

Pressure miscalibration

Depth values were calculated automatically from pressure readings by the loggers however improper calibration of the loggers resulted in negative pressure readings which were displayed as 0m depth and outside of calibration range. All data loggers had negative pressure readings as some point during deployment. These were often the first pressure reading of the deployment when the logger had been set to record data but was not yet positioned in the river. During analysis these readings were assumed to be a pressure of 0 as the logger was not yet submerged and the entire dataset was recalibrated according to this reading. For accurate and reliable pressure/depth readings in the future loggers should be calibrated at a known depth at point of deployment as this will provide a known accurate reading from which other sources of error, such as instrument ageing, can be detected.

Sensor ageing, obstruction, fouling and submersion in silt

When the loggers were deployed for long periods between data retrieval the sensitivity of the sensors decreased. The effect of this was most pronounced in the Mostyn Dock logger salinity values before and after data retrieval after three months of continuous deployment, which read 11.63psu before data retrieval and 26.28psu when redeployed 60 minutes later. The decrease in the salinity sensor sensitivity is most often caused by bio-fouling where algae and other marine organisms grow on the surface of the sensor thereby reducing measured conductivity. Thorough cleaning of the sensor between deployments should further reduce this effect. Similar issues can affect the pressure and temperature sensors and can also be controlled for.

This is a known property of the loggers and the data retrieval software accompanying the loggers can be used to correct the data with known values from independent readings taken when the logger is deployed and data is retrieved. Further error can be introduced by obstruction of the sensors either by immersion in silt or by covering of the sensor when the logger is secured into its plastic housing. The effect of silt immersion is best demonstrated by the readings from the Sandycroft data logger after the 14th November (Figure 28). Pressure readings appeared unaffected however both temperature and salinity readings stabilised, showing very little variation. Future deployment of data loggers should ensure that the loggers are positioned far enough above the river or estuary bed that silt immersion does not occur.

It appears that the Crane Wharf data logger was faulty, whereby a constant reading of -4.53m depth was recorded regardless of actual depth. Although, this could be an artefact of silting or the instrument being out of the water, however the measurement stayed exactly the same between data retrievals. Salinity values were also inconsistent with values detected by the Chester weir logger and therefore the logger should be recalibrated or replaced.

Edge effects

Readings from loggers positioned at the edge of the river or estuary may not be representative of general river conditions at that location. For instance, in tidally influenced locations where the loggers are submerged in very shallow water the water temperature may be more greatly influenced by air temperature and sunlight therefore over- or underestimating the water temperature in the river channel. This effect is particularly pronounced for the logger at Sandycroft. From March to August high and low temperatures for each month differ greatly with a largest difference of 21.4°C between the June maximum and minimum. This variation is likely to be an artefact of the shallow position of the logger at low tides. The degree of variation more closely resembles other loggers after the Sandycroft logger was repositioned in late August. Furthermore, salinity readings from a data logger positioned toward the shore may not represent the salinity of the whole river channel at that location.

Where fresh and saline water meet in estuaries the denser saline water remains beneath the fresh water with some mixing occurring where the two layers meet resulting in stratification. Consequently, a data logger positioned at the edge of the river/estuary channel taking measurements from the water surface at low tides (such as at Sandycroft and Crane Wharf) will record the fresh stratum until the rising tide raises the surface of the lower saline stratum to meet it. This effect may explain the rise in salinity at Sandycroft only once the tide reaches a depth of approximately 2 to 3m above the logger and why greater salinities are recorded at higher tides. It may be that, due to this effect, the data loggers are underestimating the salinity of the estuary channel, particularly at the channel bed where mitten crabs are potentially present.

Ideally the data loggers should have been set from independent buoys in the estuary to ensure they were set at a sub-tidal depth. Due to the cost of installing and accessing data loggers set from buoys, the loggers were set at the suitable locations accessible on foot. Consequently, at spring low tides water level dropped below the level of the Sandycroft and Mostyn Dock data loggers. The loggers are unable to read salinity unless submerged and consequently read 0psu salinity when exposed during particularly low tides. At Mostyn Dock, the low of 0psu in September was accompanied by very low depth readings therefore was most likely due to the water level dropping below the data logger rather than the presence of fresh water.

Accurate and independent salinity and temperature readings should be taken at every deployment and retrieval of a data logger. Depth of logger deployment should also be measured at the same time. Data loggers should be thoroughly and carefully cleaned between each deployment and care should be taken that nothing is obstructing the measurement instruments. These actions would allow accurate recalibration of the loggers therefore providing more accurate and reliable data.

Measurement of environmental requirements of Chinese mitten crabs

Chinese mitten crabs are a catadromous species with differing physiological requirements for temperature and salinity at each life history stage (Cohen & Weinstein, 2001). The positioning of the data loggers appears to measure the salinity gradient from fresh to salt water adequately to roughly estimate the locations of salinity thresholds which define the geographical range of each life history stage.

However, due to the sources of error detailed above, these estimations may not be wholly accurate and could be refined by addressing this error. Furthermore, data from the United Utilities and Chester, Boat Mooring data loggers were almost identical due to their close proximity. It may be more informative to reposition the United Utilities data logger to an intermediate location between Sandycroft and

Mostyn Dock as this would better resolve the transition from brackish to more marine salinities required during mating and larval development.

Future population size has been reported to be highly dependent of estuary temperatures during larval development (Blumenshine et al., 2001; Cohen & Weinstein, 2001). Temperatures recorded by the Mostyn Dock data logger are comparable to those recorded by Environment Agency data loggers in the Dee estuary suggesting that the position of Mostyn Dock logger is representative of water temperature throughout the lower estuary.

The data loggers positioned above Chester weir measure conditions at the lowest functionally fresh water location on the Dee. This supports the assertion that the weir represents the lower limit of pre-reproductive adult habitat, and confirms that Chinese mitten crab on the Dee must transition over the weir into fully freshwater conditions in order to complete their lifecycle. Consequently, data from these loggers should also be useful in investigation of potential adult migration cues. As sightings of adult mitten crabs have been reported considerably further up river of Chester, an additional data logger measuring conditions at these locations may provide complementary data and allow comparison of proposed cues throughout adult mitten crab habitat.

Deployment site access and security

The data loggers deployed at Sandycroft and Crane Wharf could only be accessed at low tide. The water height and flow rate is dependent on both tide and rainfall up river. Consequently low tide predictions for these two sites became unreliable following periods of heavy or sustained rainfall in the catchment. Accessing the Crane Wharf logger by boat became difficult following periods of heavy or sustained rainfall. All data loggers were positioned away from publically accessible areas and attached to inconspicuous chains located out of view of the public to avoid tampering or theft. These measures were successful in avoiding these issues as the loggers appear to have remained in position throughout their deployment.

Summary

Due to the catadromous life history of the Chinese mitten crab measurements of environmental variables throughout a colonised river catchment are useful for monitoring and predicting the potential impacts of a population. The use of data loggers to measure river conditions in mitten crab habitats is suitable to achieve this. Location of data loggers throughout the river and estuary was sufficient to roughly approximate the locations of the salinity thresholds for each mitten crab life history stage, and particularly confirmation that juveniles/sub-mature adults must overtop the weir in order to complete their life-cycle. This information may prove useful for future management and mitigation techniques.

7. Estimating Population Size and Trends

7.1. Estimating population size and trends objectives

Objective: Establish whether the population of mitten crabs is expanding in the River Dee and investigate whether the number of mitten crabs shows any significant change between years.

Calculating the absolute, current population size in the Dee would allow targets for control efforts to be set, which may facilitate a realistic reduction of the population. Further measurements of population size could then be used to assess the success of such measures. Unfortunately, previous attempts to calculate absolute population sizes of this species have proved difficult and were thought to be considerable underestimations (Schmidt *et al*., 2009).

In invasions of other river systems, the *E. sinensis* population has developed through three phases: initial establishment and lag phase, with a small, stable population that can last many years; followed by exponential growth and rapid population expansion; and eventual saturation (King, 2013). In order to confirm that the Chinese mitten crab population is established in the Dee, the population must be able to self-recruit by completing its life cycle in the river. If megalopae are detected in the estuary and juveniles further up river, it would suggest that the population is self-recruiting. The measurement of the abundance of these larval stages over a number of years may also provide a proxy, by which to predict changes in adult population size (Shanks *et al.,* 2010).

Objective: Determine the geographical extent of mitten crab within the Dee catchment

The project aimed to explore the geographical extent of mitten crabs within the Dee catchment. Invading mitten crab populations may have a significant ecological and economic impact throughout their range within the river catchment (Dittel and Epifanio, 2009). Understanding and predicting the current and projected range of the species on the Dee is important to gauge the extent of these impacts. To study this range, all verifiable records of mitten crab on the Dee were mapped, noting the date recorded. Using this data, inferences could be made about the potential expansion of the mitten crabs' geographical range since it was first recorded on the river. Using the outreach methodology will allow for a greater account of the geographical extent of the crab, as project staff are unable to cover the whole catchment area. Encouraging the involvement of stakeholders, especially the angling community, could provide a more thorough account of the crabs' geographical extent.

Objective: Determine seasonal variation of abundance & distribution

An understanding of seasonal variations in different life history stage distributions in the Dee catchment would enable targeted monitoring and control efforts. Data from each of the trialled methodologies were examined to identify seasonal variation in the abundances of each life history stage.

Objective: Examine environmental cues driving adult migration

For other European river catchments, management efforts have focussed on the removal of adults during downstream migration periods. Developing an understanding of the cues which drive the migration pattern would enable control efforts to be more precise. By examining correlations between changes in environmental conditions, (e.g. water temperature, moon phase, tidal conditions and day length), and the date of first capture at the NRW fish trap.

7.2. Population size & trends Methodology

This part of the project relied on the results from the other data already collected in the sampling trials. No new methodology was used for this part of the work.

7.3. Population size and trends results and discussion

Population Size

Estimation of a current, absolute population size of *E. sinensis* in the Dee could allow targets for control efforts to be set, which may facilitate a realistic reduction of the population. Further measurements of population size could then be used to assess the success of such measures. Unfortunately previous attempts to estimate absolute population sizes of this species have proved problematic and were thought to be considerable underestimations (Schmidt *et al*., 2009). It is not possible with current data to produce an equivalent estimation. However with further refinement of monitoring methodologies or pending the results of the Natural History Museum's (or future) genetic analyses (Wang & Whitlock, 2003), estimates may be possible in the future.

If it is assumed that survey effort has been approximately equal between years at Chester fish trap, variation in numbers recorded at the fish trap should reflect changes in population size. Clark (2008) used a similar approach to measure population trends in the Thames using records of the number of crabs removed from power station water inlets. It is possible that variations in numbers of crabs trapped differ due to stochastic variation in numbers entering the trap, independent of population size. It is possible that increased vigilance at the fish trap has accounted for the difference in recorded numbers between years prior to 2013, and years 2013 and 2014. If similar trends had been observed during systematic sampling of the Dee population using other methodologies (such as fyke netting and shoreline surveys) this would support the assertion that numbers trapped at the fish trap are reflective of relative population size. Other methodologies, similar to those trialled on the Dee, have successfully measured fluctuations in mitten crab relative population size between years (Gilbey *et al*., 2008; Garcia de Lomas *et al*., 2010).

Population size in their native range and in Germany fluctuate greatly (Hymanson, 1999). These fluctuations are attributed to weather events such as heavy rainfall (flooding) and drought and associated changes in water flow and estuarine salinity. Indeed, drought and increased water abstraction in the early 1990s has been proposed as the cause of the population expansion in the Thames, due to reduced flow aiding larval retention in the estuary (Herborg *et al*., 2005). Another proposed explanation for these fluctuations is that changes in estuarine water temperature strongly effect larval survival, influencing the subsequent population size of mitten crabs (Blumenshine *et al*., 2011). In more northerly river systems, such as the Dee, colder estuary winter temperatures may play a role in limiting mitten crab larval survival in certain years causing fluctuations in population size, however, future monitoring is needed to realise the full implications of this.

Establishment Phase

In order to confirm that the *E. sinensis* population is established in the Dee the population must be able to self-recruit by completing its life cycle in the river. If the post-larval megalopa stage is detected in the estuary it indicates successful larval development and infers development to juveniles in the River Dee, and therefore that the population is self-recruiting. Measurement of the abundance of these larval stages may also provide a proxy by which to predict subsequent adult population (Shanks *et al*., 2010).

No megalopae were detected in the methodological trial, so population self-recruitment cannot be proven from this year's data. However, sexually mature adults are completing downstream migration on the Dee, as shown in NRW fish trap data. Additionally, a commercial fisherman on the Dee estuary has reported catching substantial numbers of adult *E. sinensis* during mid-November. Age classes of juveniles recorded during shoreline searches and caught at the NRW fish trap also support recruitment to the population. It is possible these individuals were introduced via ballast water or migrated from a nearby, undocumented population, although this is less likely than a recruiting population.

At the NRW fish trap, records show low numbers for years 2007 to 2012, fluctuating between 4 and 21 individuals trapped per year. However, in 2013, 82 individuals were recorded, and a comparable 76 were recorded in 2014 (Figure 26). This apparent population expansion may indicate transition to the exponential growth establishment phase, although this increase is not yet as extreme as has been seen in other invaded rivers.

An additional indication of increasing population size is the increased reported range of the species. Cohen and Weinstein's (2001) model of mitten crab invasion of river catchments suggests that crabs remain concentrated in the lower reaches of a river during initial establishment. As the population increases in size juvenile crabs migrating up river must pass saturated habitat, where competition for food and favourable habitat is high, to unoccupied, suitable habitat further up river. In 2014, river stakeholders have reported mitten crabs in Almere and Rosset, further up river than any reports from previous years. This may be indicative of an expanding population, however it is also possible that the species has been present at these locations for longer and have only now been reported due to raised awareness from this project's outreach.

Particularly at colder latitudes, it is though that winter estuarine water temperature may be the main factor limiting mitten crab population growth. Colder temperatures limit larval survival and therefore the size of the subsequent population in the river (Anger 1991; Blumenshine *et al*., 2011). The data loggers deployed on the river will allow direct monitoring of river and estuarine water temperature and flow (via depth) in coming years. These data could be used to aid prediction of future population growth. Sea temperatures around the UK became warmer throughout the 20th Century and are predicted to rise by a further 2°C by 2050. This rise in temperature has already altered coastal marine ecosystems, allowing species favouring warmer waters to extend their ranges northward (Hiscock *et al*., 2005). This effect is likely to be beneficial to Chinese mitten crabs on the Dee, as the warmer waters allow increased larval survival in the estuary, and accelerate future population expansion. Mitten crab numbers should be closely monitored over the coming years to determine if, and at what rate, the Dee mitten crab population is expanding.

Geographical Extent within the Dee Catchment

Mitten crabs are typically found at the greatest abundances in the lower reaches of river catchments and the estuary, although high densities of mitten crabs have been reported upstream on many rivers. Panning (1939) reported mitten crabs on the Elbe 450 km upstream of the estuary mouth. They have since been reported in Prague 700 km upstream (Cohen & Weinstein, 2001). There are also reports 1400 km upstream on the Yangtze River (Cohen & Weinstein, 2001) On the Thames there have been reports of as far as 90 km upstream of the mouth of the river (Cohen & Weinstein, 2001). Rudnick *et al*. (2005) suggest that the potential distance of colonisation upstream is dependent on the juvenile upstream migration. The extent of the migration may be due to mitten crab density in the lower reaches of a river, with increased competition for food and refuges driving migration to less densely colonised habitat up river (Cohen & Weinstein, 2001). Population density may vary over the years in response to changing water temperatures and weather conditions.

On the Dee, mitten crabs have been recorded as far upstream as Rossett on the River Alyn (SJ 383 563) approximately 25km upstream of the estuary head at Connah's Quay. These reports in 2014, are further upstream than in previous years, possibly indicating that the species is colonising new areas further upstream or may simply be the result of increased reporting through greater awareness of the species.

The reports of Chinese mitten crabs at Almere (SJ 405 563) and Rosset (SJ 383 563) in 2014 represent the furthest known extent of Chinese mitten crabs up river. With the exception of the deployment of a fyke net at Holt in 2013, no monitoring work has been undertaken up river of these locations. Should effective adult mitten crab monitoring methodologies be developed in future, further monitoring is required on both the River Dee and River Alyn to determine the full geographical extent of the Chinese mitten crab population.

Spatial Differentiation of Life History Stages

Each *E*. *sinensis* life history stage requires different salinities present throughout a river and its estuary. Consequently, each life history stage is limited to reaches of the river that meet these requirements. Potential range of mitten crabs within river catchments in the USA have been estimated using physiological tolerances of environmental variables (Cohen & Weinstein, 2001). Using data obtained from the data loggers, and compared with records of mitten crabs in the river, rough estimations can be made about the distributions of mitten crab life history stages on the Dee.

Mating
No megalopae have been detected in estuary however sexually mature adults were caught at the estuary head (Keith Marland, *pers. comm.* 2014) during mid-November. This timing coincides with the peak mating period recorded in the Thames (Herborg *et al*., 2006), which would suggest that mitten crabs are mating and reproducing in the Dee estuary. Optimal salinity and temperature ranges for mating are reported as 10-16psu and 15-17°C. These tolerances suggest that mating should take place in the Dee at a point between the Sandycroft and Mostyn Dock data logger sites (Figure 28).

Larval development
Metamorphosis from megalopae to juvenile crabs, and settlement out of the estuary water column, can occur at salinities between 10 and 32 psu but optimal conditions are above 25 psu. This salinity range encompasses the entire Dee estuary from its mouth to approximately the Sandycroft data logger.

Juvenile development
Juvenile mitten crabs are thought to remain in brackish water for their first winter (Rudnick *et al*., 2005) which is consistent with estimated ages of crabs found during 2014 shoreline surveys below Chester weir. All *E. sinensis* found during the shoreline searches at Crane Wharf and Handbridge were below reproductive size, reinforcing that these were individuals migrating upstream. No mitten crabs were detected at the Sandycroft site on the occasions when this site was visited. This is possibly due to availability of suitable habitat such as muddy substrata and number of refugia. It is also feasible that this area signifies the lower limit of juvenile extent and future investigations could target the area for presence of megalopae at the appropriate time of year.

Non-reproductive adults
Adults were only detected at the fish trap during months of downstream migration whereas small numbers of juvenile crabs were detected throughout May to November which implies that juveniles are migrating up river past this point. If large sub-adults were being detected throughout the year at the weir fish trap (other than during the typical months of adult migration), this would indicate that conditions in the downstream of the weir were suitable to enable mitten crab to complete its lifecycle, and therefore there would be no great need for the juveniles to continue upstream.

These results are also reinforced by the finding of the data loggers which indicate that the river upstream of the weir is predominantly freshwater apart from the occasions when it is overtopped by spring tides. The maximum reported extent of the upriver migration on the Dee is represented by the records from the confluence between the Dee and the Alyn at Almere and Rosset. However, as no systematic sampling surveys have been undertaken further upstream this distance could be greater.

In China maturing crabs are typically found in vegetated lake-like, and slow moving water, while fast-moving cold rivers and streams are less suitable (Hymanson, 1999). The species is not likely to colonise far upriver of the Dee's flood plain as the river habitat becomes less suitable. Gonadal development begins in fresh water (~0 psu), however it is not clear whether gonads can fully mature in fresh water or require more saline conditions (Rudnick *et al*., 2003).

Control efforts on some other rivers have focussed on exploiting physical barriers across rivers, such as dams and weirs, to trap adult *E. sinensis* during their downstream migration (Panning, 1939). The crabs must climb over or walk around such barriers to continue their migration and will do so in predictable locations providing opportunities to place traps to remove them in large numbers. Chester weir may represent such a barrier where these methods could be used. If mitten crabs must pass up river of the weir to complete its life cycle then the weir would be an optimal location to focus control efforts of the Dee population. Although salinities below Chester Weir appear to be within the thresholds to support the entire life cycle of *E. sinensis*, it appears that many individuals are continuing upstream.

It should be noted however that in other populations a small number of individuals remain in brackish water throughout their life cycle. In native Chinese populations some mitten crabs are able to mature within one year, remaining in low salinity areas of the estuary. Maturation to sexual maturity is thought to accelerate in saline conditions but results in smaller crabs with reduced fecundity (Hymanson Z., 1999), although, this has not yet been reported in any European populations.

Annual Patterns of Mitten Crab Distribution

Seasonal variations in Chinese mitten crab abundance differ for three broad stages of its life history.

Larval stages in the estuary

No megalopae were detected during the 2014 megalopa sampling trial so no firm conclusions can be drawn about their annual patterns of abundance. However, the environmental conditions required for mitten crab reproduction and larval development are well documented for other populations (Anger., 1991; Blumenshine *et al*., 2011; Rudnick, *et al*., 2005). Consequently, general predictions can be made about seasonal abundance in the Dee using environmental data from the Mostyn Dock data logger and other Environment Agency loggers situated in the estuary.

Suitable estuary water temperature (>12°C) is crucial for larval survival and development (Anger, 1991, Blumenshine *et al*., 2011). Water temperature in the Dee estuary between October and May is likely to exclude the presence of mitten crab larvae due to low survival below 12°C. Mating typically takes place from mid-November after which females spawn and retain the eggs until the embryos develop and hatch. Under 17°C laboratory conditions embryos take approximately 30 days to develop from fertilisation to hatching but embryo development is thought to be slowed in colder temperatures (Rudnick *et al*., 2005). This implies that most larvae will hatch during the earlier months of the Dee’s more favourable temperature window. Coupled with a larval development time of 18 to 74 days from hatching to metamorphosis into juvenile form (Anger, 1991), this would suggest that larval stages are likely to be at greatest abundance in the Dee estuary between May and July.

Upstream migration of juveniles

Following metamorphosis, juvenile crabs settle out of the estuary water column and begin migration up river to fresh water habitats. Greater numbers of juvenile mitten crabs were detected during July shoreline surveys than the later autumn surveys. Juvenile crabs were detected at the fish trap from May until November at the latest. These results are consistent with a comparison of reported juvenile migration rates and predicted settlement period in the Dee estuary. (Hymanson *et al*., 1999, Cohen & Weinstein, 2001). Juvenile growth and activity is possible from minimum water temperature of 7^{o}C (Rudnick *et al*., 2005). As measured by all data loggers' water temperatures above correspond to the months throughout the year that mitten crabs are detected at the fish trap.

Downstream migration of reproductive adults

Downstream migration of reproductive adults from freshwater habitats occurs during the same period annually across all populations (Panning, 1939; Hymanson *et al*., 1999; Morrit *et al*., 2013). The migration occurs from August to October and usually peaks during September. The timing of records from the fish trap are consistent with this period. Mating is thought to occur as in other populations in the upper estuary from mid-November onwards (Cohen & Weinstein, 2001). Reports from a fisherman at Connah's Quay of catching adult mitten crabs of both sexes and adult size starting in mid-November appear to indicate the arrival of mating adults to the Dee estuary.

Environmental Cues Driving Migration

Downstream migration is consistent throughout all mitten crab populations (Rudnick *et al*., 2005) and highly predictable. This makes the migration an excellent opportunity for population monitoring and control efforts. Despite the fact that the timing of the migration is well documented across both native and invasive mitten crab populations there has been no definitive study identifying the migration cue. There is, however, much speculation on the topic. Anger, 1991 (cited in Cohen & Weinstein, 2001) suggested downstream migration of *E. sinensis* is triggered by hormones released by the developing gonads once the crabs reach a minimum size.

A similar cue has also been suggested in the closely related, and behaviourally similar, *Eriocheir japonicas* (Kobayashi, 1995). A minimum reproductive size of 30mm carapace width below which no migration response has been reported (Rudnick *et al*., 2005) and no reproductively mature crabs below 30mm carapace width were recorded at the Chester fish trap. Although gonad maturation above a size threshold may explain the timing of the downstream migration in mitten crab life history, it does not explain the relatively simultaneous (within approx. 8 weeks) annual migration. Consequently the ultimate stimulus is thought to be environmental (Rudnick *et al*., 2005).

Hydrological factors such as changes in water temperature or increased rainfall have been proposed. The first adult mitten crabs at the fish trap in 2014 was recorded one month after the peak river temperature recorded by Chester data loggers. Peak daily average water temperature was recorded on the 28th July at 21°C. The temperature on the day the first mitten crab recorded at the fish trap during the migration period was 15°C (Figure 29). This drop in water temperature of 6°C is a similar temperature change to the reported drop from 25°C to 20°C reported by Jin & Li, 1998 (cited in Cohen & Weinstein, 2001).

In France and Germany downstream migration has been associated with increasing rainfall and river flow (Cohen & Weinstein, 2001). Met office regional monthly rainfall averages (metoffice.gov.uk, accessed 02/02/2015) for North West England and North Wales indicate an increase in rainfall from 69.3mm in July to 140.2mm in August 2014. This may suggest that increased rainfall is a possible migration cue however the average drops to 15.1mm for September so rainfall would not account for peak numbers during this month unless only a brief period of rainfall is required.

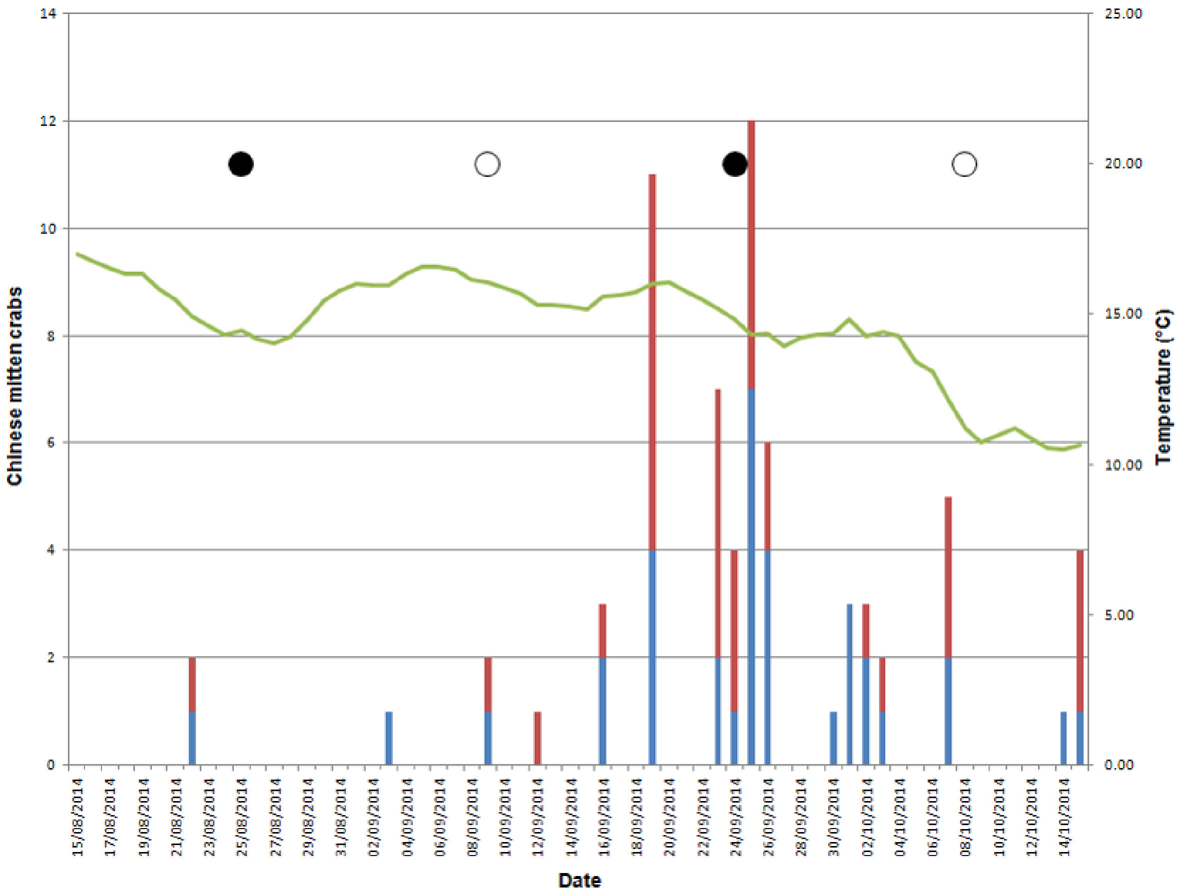

Figure 29. Numbers of male (blue) and female (red) mitten crabs recorded at Chester during the 2014 downstream migration period.
Daily average water temperature (green) and dates of new (black circle), and full moon (white circle) are plotted as potential migration cues

Weather patterns vary globally whereas the timing of mitten crab migration remains fairly consistent, leading some to suggest that a more universal cue such as photoperiod or lunar cycle is more likely (Rudnick *et al*., 2005). The first adults were recorded at the fish trap approximately 2 months after the summer solstice (on the 22nd August) when day length had dropped from 16h 58m to 14h 18m, peak numbers occurred a month later when day length was 11h 59m. The first record of an adult mitten crab at the fish trap during the 2013 migration was also made on the 22nd August: the same interval between the solstice and first record. Peak numbers were approximately one week earlier in 2013 than in 2014. However, this difference in the dates of peak migration could be due to other factors. For instance, if the population has colonised further upriver in 2014, then the later peak migration could be due to the time taken to migrate additional distance.

Morrit *et al*. (2013) reported peak numbers of migrating mitten crabs were associated with full moon periods. First adult mitten crabs recorded at the fish trap 3 days before the new moon on the 25th August. Peak numbers recorded at the fish trap coincided with the new moon on the 24th September which may suggest a lunar migration cue. However no such association was observed during the 2013 migration. With current data the adult downstream migration cue for Chinese mitten crab on the river Dee remains unclear. Comparison of proposed migration cues with additional year's data may allow more meaningful conclusions to be drawn.

Introductory Pathways to the Dee

The most important introduction pathway of Chinese mitten crabs to river catchments is translocation of larvae and juveniles in ship ballast water (Cohen & Carlton, 1997). Active shipping between the Dee estuary and other areas in continental Europe with known populations of Chinese mitten crabs (King, 2013) is therefore most likely responsible.

DNA samples were taken from Chinese mitten crabs trapped at the NRW fish trap in 2013 to be analysed by the Natural History Museum to determine the potential source population of the species on the Dee. The analysis is currently awaiting collection of European samples before it can be completed. These are expected to have been obtained by March 2015 so analysis can begin shortly thereafter (Paul Clark, pers. comm., 2015).

The River Dee has the only known population of Chinese mitten crabs in Wales and North West England, although there are reports of the species on the Mersey. It is possible that it may act as a source population for the colonisation of other, nearby, rivers.

Hanfling *et al*. (2002) found low genetic differentiation between populations within continents which they attributed to extensive larval drift allowing gene flow between populations. Chinese mitten crab larvae have the potential to drift up to ≈60km depending on marine currents (Herborg *et al*., 2006). These findings indicate that nearby river catchments, such as the Mersey, may be at risk from natural colonisation by the species. Should an effective larvae or megalopa sampling methodology be developed, the coast between the two estuaries could be monitored to assess whether the populations are linked by larval drift.

The Dee is also connected to the Mersey and other rivers by an extensive canal network (King, 2013), by which the Dee population may also spread to other nearby catchments.

As an alternative to monitoring coastal megalopae, a genetic study examining differentiation and gene flow between the two populations could be conducted to determine if they are linked. A genetic study would determine whether the populations are linked but may not be informative as to the linking pathway (i.e. larval drift or dispersal through inland waterways)

Translocation of Chinese mitten crabs has also been raised as a concern in commercial mussel seed harvest from the Dee as larvae and small juveniles could feasibly be accidently transported with the mussels. As a consequence mussel seed harvest from the Dee estuary has been limited to August when the abundance of benthic mitten crab life history stages should be low. Maintaining public awareness of the species and associated problems is important in preventing further spread.

8. Conclusions

Outreach has raised the profile of the species with stakeholders on the river however continued public engagement is required to maintain awareness of the importance of potential Chinese mitten crab impacts on the Dee. Much of the outreach work has been conducted in tandem with the Dee Invasive Non-Native Species project which may be a suitable vehicle to maintain ongoing public awareness of the species and its impacts.

Shoreline surveys are potentially an effective method to monitor mitten crabs below Chester weir. The methodology is simple to conduct and easily quantifiable. Use of artificial refugia as a trapping method could be investigated.

As yet there is no effective and reliable method of trapping adult mitten crabs in the River Dee. Despite the lack of mitten crab in the modified fyke nets in the current study, this method may still be viable with revised equipment, more suitable deployment locations or in a catchment with higher population numbers.

The megalopa collectors were not successful in detecting mitten crab larval stages in the estuary but this may require increased sampling effort across a wider range of locations. Alternative sampling methods such as plankton trawling (Blumenshine *et al*., 2001) could also be investigated. This method could be especially useful for determining risk of translocating the species from the Dee to other catchments via shipping or mussel seed harvest.

Incidental trapping at the NRW fish trap, Chester, continues to be the most effective tool for monitoring the mitten crab population on the Dee. Further investigation into the direction from which mitten crabs enter the trap, and the conditions which attract the species into the trap would provide useful insight for future management at the site. This information could then inform modifications to the fish trap to increase its effectiveness. For instance, reduction of the bar spacing at the opposite side from which crabs enter, or installation of dedicated refuges within the trap, may increase retention of crabs.

The data loggers have been informative regarding geographical and temporal limits of life history stages and investigation of migration cues. Use of loggers in future will continue to be informative but requires adjustment of protocols for reliable and accurate data. Additional loggers would provide additional resolution to data but are not required to meet current objectives of the project.

As limited useful data was obtained during these methodological trials, assessments made in this report are largely speculative and not scientifically robust. The data obtained may however, act as a baseline for future research into the state of Chinese mitten crabs on the River Dee.

The investigation of downstream migration cues is largely inconclusive and requires a longer running study comparing potential migration cues and records over several years or a study of potential cues across migrations in multiple populations.

The River Dee Chinese mitten crab population size and geographical range appears to be increasing, although stochastic variations have been recorded in other populations and the population appeared stable between 2013 and 2014. Other than occasional reports from river stakeholders, little is known about the current extent of Chinese mitten crabs upriver. It would be useful to continue to develop monitoring methods, such as fyke netting and possibly the application of eDNA sampling to assess the extent of the population expansion upstream from Chester.

The population should be closely monitored in coming years, its potential impacts assessed, and plans for population management be developed. Currently, there has been no formal assessment of the potential ecological and economic effects Chinese mitten crabs will have on the Dee. As the River Dee has been designated a Special Area of Conservation, due to populations of a number of nationally and internationally important species, such a study should determine the effects of Chinese mitten crab on these species as well as wider river ecosystem. Furthermore the Dee is an important economic resource, providing water to local communities and industry as well as sustaining some commercial fisheries. Mitten crabs are documented to affect both water abstraction and fisheries consequently the potential risk to these industries should be studied and any other economic or social effects identified.

Until results of the genetic analysis from the Natural History Museum are complete the origin of *E. sinensis* on the Dee remains unknown. However, shipping operators to the estuary could be contacted and measures to avoid further introductions or translocations could be explored. As ballast water has been identified as the primary introduction pathway for Chinese mitten crab, best practice guidelines for control and management of ballast water should be promoted. However shipping operators are currently under no legal obligation to manage ballast water to prevent the spread of invasive species as the UK has not ratified the International Convention for the Control and Management of Ships Ballast Water & Sediments (International Marine Organisation, 2015)

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10. Appendices

10.1. Further outreach materials

Project poster (English and Welsh)

Chinese mitten crab identification cards

10.2. Sampling protocols

Shoreline survey protocol

- Divide the survey site into equal sections according to the number of pairs of surveyors.
- Each section will be surveyed by a minimum of two surveyors; for health and safety, and also one to record number of rocks checked and data on crabs, the other to physically check under refugia.
- Carefully lift suitable refugia and check the substrate beneath for crabs.
- Record the species, sex (male/female/unknown), carapace width (in mm), status (alive/dead), time found and any additional comments for each individual crab. This data should be recorded for all crab species.
- Place all crabs into containers. Native species should be kept separate in a separate container from *E. sinensis* for release after survey. *E. sinensis* found should be placed into a secure container to be removed from site.
- Once all crabs have been recorded from beneath a refuge it should be replaced to its original position and marked as checked.
- Record the number of refugia checked as they are turned as a tally.
- Continue until all suitable refugia in the section have been checked.
- Record time finished.
- Release native species
- Measure and record total area surveyed either by walking the perimeter with a GPS device or by measuring the width and length of the site in meters and multiply to approximate area.

Fyke Net Protocol - 2014

Fyke net deployment

- Tie the rope attached to the net wing to the metal stakebar driven into river bank with the net in the boat.
- Launch the boat and row toward the opposite bank feeding out rope and wing until within a few meters of the opposite bank, level with the bar.stake.
- Drop the anchor attached to other wing then row back towards the original bank feeding out the anchor-side wing while drawing in the opposite wing and rope attached to the bank.
- Continue to feed out net including the cone and anchor until reaching the bank
- Tie the excess rope to the barstake so the wings form a 'V' down river.
- In the boat, lift the top edge of the net and follow it to the far end of the anchor-side wing, ensuring there are no twists.
- Return to the conical net and lift the attached anchor into boat
- Row down river with anchor until the conical net is fully extended and the wings taught. Then drop the anchor
- Record date and time

Checking the net

- Follow the top edge of the wing until reaching the conical net
- Lift the cone into the boat, carefully checking each section for crabs and by-catch
- Record the time checked
- Any trapped animals should be removed from the net by untying the drawstring at the end of the cone.
- Record species and status (alive/dead) for all trapped specimens
- Living by-catch should be released immediately after recording; *E. sinensis* should be placed into a secure container to be removed from site.

Net retrieval

- Lift anchor on the far wing and pull the net into boat, lifting cone and anchor, until reaching other bank.
- Record the time net was retrieved.
- On land, re check the net for any trapped animals or plant matter before being soaking in Virkon and allow to air dry.

Fyke netting licences

creating a better place

Environment Agency

Rhian Hughes
North Wales Wildlife Trust
Pisty Farm
Longbarn
Nercwys
Mold
CH7 4EW

Our ref M/SO/09092013/AJE1

Your ref

Date 06/09/2103

Dear Rhian,

AUTHORISATION TO USE FYKE NETS FOR SCIENTIFIC RESEARCH IN THE ENGLISH *STRETCH OF THE* RIVER DEE.

The person named above is authorised in accordance with Section 27A Salmon and Freshwater Fisheries Act,(as amended by the Marine and Coastal Act 2009,) to fish for Chinese Mitten Crab (*Eriocheir sinensis)* in waters under the jurisdiction of the Environment Agency.

The authorisation holder has been granted the use of up to **4** single fykes (one cod end =one fyke)

NGR of each net : SJ 40712 65814, SJ 41440 61945, SJ41833 60054, and SJ40432 55270.

Authorisation valid from: 06 SEPTEMBER 2013

Authorisation valid to: 09 DECEMBER 2013

Tag Numbers : M/SO/09092013/AJE1 **1,2,3,4**

Additional person(s) authorised:

North Wales Wildlife Trust: TimYardley, Mike Klymko ,Rob Gardiver ,Abbey Pennington.

Natural Resource Wales : Ben Wray, Gabe Wyn , Rowland Sharp, Maggie Hatton-Ellis, Matt Ellis.

Cheshire Wildlife Trust: Lyn Byrne

Chester Zoo: Sarah Bird.

The authorisation holder is authorised to fish the English part of the River Dee. No fixed engines or traps to be used in the River Dee without prior consent from the landowner.

The authorisation holder is subject to the conditions that are attached in 'Schedule 1: Standard authorisation conditions (with the exception of Condition 11). It is an offence to fish in contravention to these conditions

The Environment Agency can revoke this authorisation at any time.

The following conditions apply;

1, Notice of the commencement of sampling must given to this office (01925542290) or the catchment officer (Paul Blake 07768730258 or Trevor Oldfield 07824538082) 24 hours in advance.

2, Otter guards provided must be securely fitted to all fyke nets.

3, Any fish captured during this study, including eels, must be returned to the water alive. A written record of species and numbers of by catch caught in individual traps shall be kept. If any by-catch is caught this needs to be reported to The Environment Agency, on a daily basis by text or email (07768276922 or andy.eaves@environment-agency.gov.uk.)
4, Fyke nets are to lifted and emptied daily and left no longer than 24 hours.

5, Any changes to the agreed method statement, REF: rh1, as must be approved beforehand with the Environment Agency,.

6,Any Chinese Mitten Crabs caught must be destroyed and not sold.

Yours sincerely,

N Mercer

Nick Mercer
Team Leader
Fisheries and Biodiversity Team

01925542834

The Environment Agency, Richard Fairclough House, Knutsford Road, Latchford, Warrington WA4 1HT

Megalopae sample collection protocol

- Check spring high tide times and plan sample collection to coincide with high tide.
- Contact Mostyn Dock Authority at least 24 hours before planned sample collection to arrange access and check that the dock is not in use at the planned sample collection time.
- Once on site, ensure all staff are wearing life jackets. At Mostyn all staff must also be wearing high-visibility jackets and safety boots.
- At Mostyn, fix the ladder to the railings next to the collector attachment point.
- One staff member should decend the ladder to the attachment point (Mosyn), or boat (Connah's Quay). The other staff member should remain above with a throw line and be ready to take the collectors.
- Pull up the chain to which the collectors are attached and pass up to the waiting staff member. At Connah's Quay the chain must be detached from the boat to allow this. At Mostyn the chain should be attached to the railings using a cable tie.
- Using the cutters, remove three of the cable ties holding one end of each collector closed.

For each collector:

- Empty the collector material, and any sediment, into a separate 2.5l bucket.
- Measure 500ml of filtered seawater into the measuring jug.
- Rinse the collector housing over the bucket with some of the 500ml of filtered seawater to dislodge any remaining crabs or megalopae.
- Transfer all the contents of the bucket to a sample pot and fill with the remainder of the 500ml of filtered sea water.
- Using the indellible marker, record the location, collection date, collector material colour and staff member initials, onto a sheet of waterproof paper and insert into the sample pot.
- Fit the lid to the sample pot, then mark the outside of the pot with the same information as the waterproof paper.
- Unfold three fresh scourers of the same colour and place into the open collector.
- Record the colour scourer used. Each collector should contain only one colour of scourer. All three collectors deployed at a site should contain a different scourer colour each.
- Reattach the end of the collector using cable ties.
- Once this process is complete for all three collectors, pass the collectors back down the the waiting staff member.
- Redeploy the collectors to their original position. At Connah's Quay ensure that the chain is securely attached to the boat.
- Record date and time of redeployment.
- Once samples have been retrived from both sites, return to the lock-up to fix the samples.
- In a well ventilated area, wearing safety goggles and gloves, to each sample pot add: 500ml of 10% formalin solution to fix the samples and 1tsp of borax powder to buffer.
- Thoroughly rinse away any spillages with fresh water.

Data logger Retrieval Protocol

- Row to the data logger attachment point at sites where required.
- Lift the chain and attached logger
- Remove logger from the chain by cutting the cable ties
- Mark the logger's position on the chain with a cable tie
- Remove data logger from its protective plastic housing by removing all cable ties and plastic bolt and locking nuts.
- Clean any build-up of sediment from the data logger taking particular care that the flat end of the logger is clean (Be careful – this is the pressure sensor)
- Connect the cable between the communication box and tablet then connect the battery to the communication box and turn on the tablet
- Insert data logger into communication box
- Load the SeaStar program and run the connection wizard.
- Download data
- To reset the logger select Edit > New Measurement Seq. Def. then select single recording interval.
- Set start time to 15 minutes after the current time to ensure logger is returned to the water before it starts recording.
- Set measurement interval time to 15 minutes then press 'OK'
- Select Recorder > Start New Measurement Sequence
- Allow new settings to upload to logger
- Remove logger from connection box **before** clicking 'OK'
- Shut down tablet and disconnect all cables
- Replace logger into protective housing and secure with the bolt and locking nuts
- Reattach logger to its original position and orientation on the chain using three cable ties through the holes in the protective housing. Cable ties **must not** cover or put pressure on the flat end of the logger.
- Lower logger and chain carefully back into the water into its original position.

10.3. Laboratory protocols

Megalopae Filtering Protocol

Wear gloves, lab coat and safety goggles. If any tears appear in the gloves wash hands and replace them right away. Perform all work with the formalin in a fume hood to avoid inhalation. Set up is shown in *Figure38.*

1. Label 250ml bottle with the details of where, when and what colour mesh (plastic scourer collection material) was used.
2. In a fume hood open the container and remove the water proof paper. Place in the 250ml bottle.
3. Remove all 3 pieces of mesh and place in a bucket, fill until mesh is submerged, but not so full that it can't be easily picked up and poured.

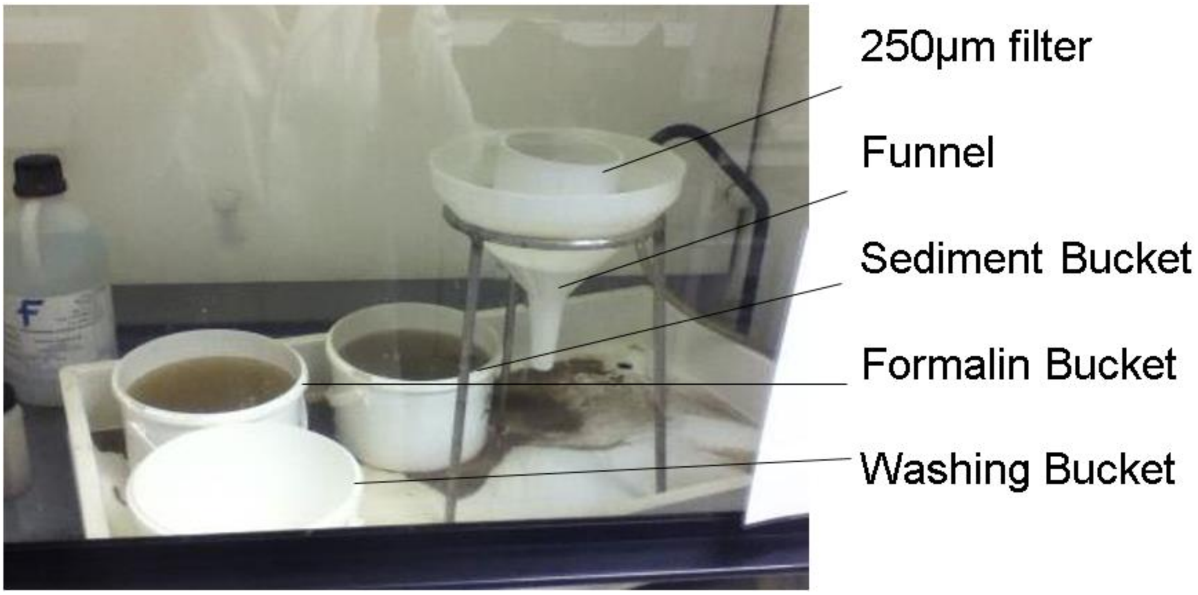

Figure 8. The set up for filtering of the megalopa

Set up a funnel with a 250μm filter in it over a bucket. Have 3 more buckets to hand.

4. Using a 250μm filter, pour the formalin from the container through the filter collecting all the formalin in the bucket. If there is a lot of sediment in the bottom of the container stop pouring and switch the formalin bucket with a new empty one.
5. Then fill with water and filter until the original container is empty.
6. All sediment which has been filtered will be kept in a bucket to prevent the drains from getting blocked and will be disposed of at the end.
7. In another bucket place one of the pieces of mesh. Rinse with water and using your hands, wash like you would hand wash clothing.
8. Place the mesh back in with the other pieces. If the water left in the bucket is full of sediment, filter over the sediment bucket. If not, can just filter straight to the drain.
9. Continue washing the mesh until there is nothing left on it. Then search all along the outside and inside of the mesh to ensure there is nothing left holding on.
10. Repeat steps 9 to 11 for the other pieces of mesh. If the filter gets blocked with sediment, using the round end of a pipette, stir circles on the mesh until all the water goes through.
11. Before placing contents left on the filter in the 250ml bottles, rinse to remove as much sediment as possible.

12. Using the hose, move the entire sample to one side of the filter. Place the bottle underneath and bang the filter against the sides of the funnel to encourage the sample to fall out. Then place the filter in one of the buckets to ensure nothing is lost. Using the pipette push the sample through the funnel into the bottle. The hose can also be used to do this, however as little water as possible should go into the 250ml bottle.
13. Using the hose over the bucket, not over the bottle, move what is left of the sample to one side of the filter. Place the filter at an angle on the funnel so you can spray the site where the last of the sample is.
14. You don't want too much water going into the bottle so make sure the hose is on low.
15. If there is any matter which still hasn't gone into the bottle use the pipette to place it in the bottle.
16. When everything is in the bottle fill up with formalin.
17. Rinse the outside of the bottle with fresh water to ensure no traces of formalin.
18. This should take 30-45 minutes, depending on the amount of sediment.
19. After all steps have been followed, check that the location, date and time and colour have all been recorded on the outside of the bottle.

Figure 9. The total set up for analysis showing the directing microscope, Eppendorf tubes, filtered sea water, container for sorting through the samples, Petri dishes, pipettes and tweezers.

Analysis Protocol

1. Pour the contents of the 250ml bottle through the 250µm filter and rinse thoroughly to ensure no formalin is left in the sample.
2. Then empty into a container and use water to ensure nothing is left on the filter.
3. Set up a lamp to shine over the container and pick through the sample extracting all crabs and megalopa.
4. Place them in separate Petri dishes to allow for easier analysis later.
5. Once there are no longer any crabs or megalopa left in the sample use a microscope to identify the species.
6. Sort the megalopa into those whose rostrum is visible from above and those whose isn't. Using identification keys determine how many organisms there are of each species.
7. If organisms are too damaged to identify keep them, but record them as unknown or damaged.
8. Store megalopa with visible rostrums and any crabs other than Carcinus in an Eppendorf tube and label it clearly. Store all megalopa without visible rostrums in a separate Eppendorf tube to make it easier to check later if needed. Use IMS to keep the samples preserved.
9. Using identification keys, identify any unknown crabs and all megalopa.
10. After all steps have been followed, ensure that location, date and time and colour are recorded in the lab notebook and that the Eppendorf number where the megalopa are stored in are also recorded in the notebook.

10.4. Additional megalopae data

Data reproduced here courtesy of Kate Cooper, Bangor University.

Counts of species and life history stages of Decapoda sampled by megalopa collectors deployed at Connah's Quay, 2014.

Month	Date	Colour	Eriocheir Megalopa	Carcinus maenas crab	Atelecyclus rotundatus	Pisisdia longicornis crab	Carcinus maenas megalopa	Pisidia longicornis meglaopa	Cancer pagurus megalopa	Anapagurus megalopa	Anapagurus crab	Unknown (Damadged)	Eppindorf number
June	2/6/14	AC	0	0	0	0	0	0	0	0	0	0	N/A
June	2/6/14	Blue	0	0	0	0	0	0	0	0	0	0	N/A
June	2/6/14	Green	0	0	0	0	0	0	0	0	0	0	N/A
June	20/6/14	Red	0	59	0	0	4	0	0	0	0	2	20
June	20/6/14	Yellow	0	51	0	0	1	0	0	0	0	0	18
June	20/6/14	Yellow	0	52	0	0	4	0	0	0	0	0	19
June	30/6/14	Blue	0	206	0	0	69	0	0	0	0	1	26
June	30/6/14	Red	0	119	0	1	57	0	0	0	0	0	24
June	30/6/14	Yellow	0	113	0	0	50	0	0	0	0	6	25a and 25b
July	16/7/14	Blue	0	494	0	0	178	0	0	11	0	28	8a and 8b
July	16/7/14	Red	0	555	0	0	222	0	0	0	0	8	7a and 7b
July	16/7/14	Yellow	0	395	0	0	137	0	0	3	1	9	3a and 3b
August	1/8/14	Blue	0	281	0	0	4	0	0	0	1	1	32
August	1/8/14	Red	0	343	0	1	1	0	0	0	0	0	30
August	1/8/14	Yellow	0	292	1	0	5	0	9	1	0	0	31
August	15/8/14	Blue	0	131	0	0	14	0	0	0	1	0	33a and 33b
August	15/8/14	Red	0	113	0	0	16	0	0	0	0	0	35
August	15/8/14	Yellow	0	189	0	0	22	0	0	0	0	1	34
August	29/8/14	Green	0	0	0	0	0	0	0	0	0	0	N/A
August	29/8/14	Red	0	0	0	0	0	1	0	0	0	0	N/A
August	29/8/14	Yellow	0	0	0	0	0	0	0	0	0	0	N/A
September	12/9/14	Green	0	6	0	1	0	0	0	0	0	0	42
September	12/9/14	Red	0	22	0	0	3	0	0	0	0	1	2
September	12/9/14	Yellow	0	17	0	0	5	0	0	0	0	0	43
Average			0	143.25	0.041666667	0.125	33	0.041666667				2.375	27.38461538
Total			0	3438	1	3	792	1	9	15	3		

Counts of species and life history stages of Decapoda sampled by megalopa collectors deployed at Mostyn Docks, 2014.

Month	Date	Colour	Eriocheir Megalopa	Carcinus maenas crab	Atelecyclus rotundatus	Pisisdia longicornis crab	Carcinus maenas megalopa	Pisidia longicornis meglaopa	Cancer pagurus megalopa	Anapagurus megalopa	Anapagurus crab	Unknown (Damadged)	Eppindorf number
May	23/5/14	AC	0	144	0	0	26	0	0	0	0	3	9
May	23/5/14	AC	0	86	0	0	2	0	0	0	0	1	10
May	23/5/14	Red	0	153	0	0	16	0	0	0	0	1	11
June	2/6/14	AC	0	330	0	0	68	0	0	0	0	1	12a and 12b
June	2/6/14	AC	0	143	0	0	40	0	0	3	0	5	14
June	2/6/14	Red	0	413	0	0	50	0	0	2	0	1	13
June	20/6/14	Blue	0	152	0	0	4	2	0	0	0	0	16a and 16b
June	20/6/14	Green	0	122	0	0	4	0	0	0	0	0	15
June	20/6/14	Red	0	94	0	0	8	0	0	0	0	1	17
June	30/6/14	Green	0	234	0	9	63	14	0	3	3	5	23a and 23b
June	30/6/14	Red	0	418	0	5	42	8	0	0	0	0	21a and 21b
June	30/6/14	Yellow	0	145	0	4	14	8	0	0	0	0	22
July	16/7/14	Blue	0	215	0	20	11	12	0	0	0	1	5a and 5b
July	16/7/14	Red	0	116	0	10	14	5	0	1	1	2	4a and 4b
July	16/7/14	Yellow	0	137	0	25	11	16	0	1	0	0	6a and 6b
August	1/8/14	Green	0	54	1	35	5	6	2	0	0	2	27
August	1/8/14	Red	0	128	0	15	13	7	0	0	1	0	28
August	1/8/14	Yellow	0	90	0	13	2	7	0	0	0	0	29
August	15/8/14	Green	0	23	0	5	3	2	0	0	0	0	36a and 36b
August	15/8/14	Red	0	47	1	5	5	3	1	0	0	2	37
August	15/8/14	Yellow	0	0	0	0	0	0	0	0	0	0	N/A
August	29/8/14	Blue	0	21	2	0	11	2	0	0	0	1	39
August	29/8/14	Green	0	50	5	4	14	7	0	0	1	0	38
August	29/8/14	Yellow	0	25	3	8	6	5	0	0	0	0	40
September	12/9/14	Blue	0	20	2	9	2	5	0	0	0	4	1
September	12/9/14	Red	0	32	1	9	10	11	1	0	0	0	45
September	12/9/14	Yellow	0	24	3	8	2	7	0	0	0	0	44
Average			0	126.51852	0.666666667	6.814814815	16.51851852	4.703703704				1.111111111	24.38888889
Total			0	3416	18	184	446	127	4	10	6		

Data Archive Appendix

Data outputs associated with this project are archived on server–based storage at Natural Resources Wales.

The data archive contains:

[A] The final report in Microsoft Word and Adobe PDF formats.

[C] A series of spreadsheets containing the raw data for species recorded as part of the current report

[F] A full set of images produced in jpg format.

Metadata for this project is publicly accessible through Natural Resources Wales' Library Catalogue http://libcat.naturalresources.wales/webview/ (English Version) and http://libcat.naturalresources.wales/cnc/ (Welsh Version) by searching 'Dataset Titles'. The metadata is held as record no's116497 and 116497.

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